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

Policy Number: CS152LA.~~N~~O
Effective Date: ~~January 1, 2023~~ TBD

➲ Instructions for Use

Certain content mandated by Louisiana Department of Health

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Application

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

Coverage Rationale

State-Specific Criteria

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

Genetic Counseling

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

- Obtaining a structured family genetic history
- Genetic risk assessment; and
- Counseling of the enrollee and family about diagnosis, prognosis, and treatment (LA MCO Genetic Counseling and Testing, page 112)

Breast Cancer

Coverage of Oncotype DX, Breast Cancer Assay for the Determination of Breast Cancer Prognosis:
Coverage Criteria:

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

Gene expression profiling as a technique of managing the treatment of breast cancer is considered investigational and not medically necessary when a gene profiling test other than Oncotype DX Breast Cancer Assay is being used, including but not limited to:

- **Breast Cancer Gene Expression Ratio (also known as Theros H/ISM)**
- **Breast Cancer IndexSM**
- **Insight® DX Breast Cancer Profile**
- **MammaPrint®**
- **Mammostrat**
- **Oncotype DX DCIS**
- **Pam50 Breast Cancer Intrinsic ClassifierTM**
- **The 41-gene signature assay**
- **The 76-gene "Rotterdam signature" assay**
- **THEROS Breast Cancer IndexSM**

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

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

Additional Non State-Specific Criteria

Solid Tumor Testing

~~Breast Cancer~~Gene Expression Profiling (GEP)

~~The use of one of the following Gene Expression~~ GEP Ttgene expression tests ~~- MammaPrint®, Oncotype Dx® Breast, Prosigna® PAM-50 Breast Cancer Prognostic Gene Signature Assay~~ (formerly ,PAM-50) ~~Breast Cancer Index ™~~

~~(BCI) and EndoPredict® – is proven and medically necessary when used to inform to make a treatment decisionsdecision regarding adjuvant chemotherapy in females or malesindividuals with non-metastatic breast cancer in the following situations:~~

- ~~Newly diagnosed (within the last 6 months) when all of the following criteria are met:~~
  - ~~Lymph node negative (including lymph nodes with micrometastases no greater than 2 mm) e or 1-3 positive axillary lymph nodes diagnosed via surgical resection of tumor (not biopsy); 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); 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~~

~~or~~

- ~~Currently receiving adjuvant hormonal therapy (e.g., Tamoxifen or an aromatase inhibitor) for a breast cancer diagnosed within the prior six years when all of the following criteria are met:~~
  - ~~Individual has not had prior Gene Expression Testing; and~~
  - ~~Hormone receptor-positive (estrogen receptor positive, progesterone receptor positive or both); and~~
  - ~~HER2 receptor negative; and~~
  - ~~Individual and treating physician have had a discussion prior to testing regarding the potential results of the test and determined to use~~

~~the results to guide a decision regarding extended adjuvant hormonal therapy~~

~~The Uu~~Use of more than one Gene Expression Test ~~GEP~~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.~~

~~, which can be used once in the evaluation of the role of extended endocrine therapy in a breast cancer that may have already had GEP to determine the role of adjuvant chemotherapy..~~

~~Gene Expression Testsexpression 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 GEPexpression profiling assays for breast cancer indications (including ductal carcinoma in situ [DCIS]) or treatment decisions breast cancer treatment other than those previously described as proven covered are unproven and not medically necessary. Such tests may include, but are , including but not limited to:~~

- ~~BluePrint (also referred to as ""80-gene profile")"~~
- ~~Breast Cancer Gene Expression Ratio (also known as Theros H/I)~~
- ~~®Oncotype DX Breast DCIS Score® test~~
- ~~The 41-gene signature assay~~

~~The 76-gene ""Rotterdam signature"" assay~~

Lung Cancer

Molecular profiling of solid tumor tissue in metastatic non-small cell lung cancer is proven and medically necessary when all of the following criteria are met:

- **No prior molecular profiling has been performed on the same tumor; and**
- **One of the following:**
  - **The multigene Next Generation Sequencing (NGS) panel selected has no more than 50 genes; or**
  - **Individual meets criteria for companion diagnostic testing below**

Liquid B~~b~~iopsy (cell-free DNA [cfDNA] or circulating tumor DNA [ctDNA]) ~~(circulating tumor cell free DNA)~~ molecular profiling tests of non-small cell lung cancer are proven and medically necessary when the following criteria ~~is~~ are 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 tumor tissue testing is not feasible; 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~~**One of the following:**
  - The **multigene NGS** ~~test~~**panel** selected has no more than 50 genes; ~~and~~**or**

- ~~o~~ Individual meets criteria for companion diagnostic testing below
- o

Prostate Cancer Gene Expression Profiling (GEP)

The use of the 17 gene mRNA score (e.g., Oncotype DX® Genomic Prostate Score [GPS]) is proven and medically necessary for individuals with biopsy-proven, untreated, localized adenocarcinoma of the prostate (no clinical evidence of metastasis or lymph node involvement) when:

- Test is ordered by a urologist or medical oncologist; and
- Results will be used to assist with treatment decision-making when the individual has not yet received treatment for prostate cancer and is a candidate for either active surveillance or definitive therapy and all of the following:
  - Life expectancy greater than 10 years; and
  - Risk group (as per NCCN) is one of the following:
    - Very-Low-Risk Prostate Cancer; or
    - Low-Risk Prostate Cancer: or
    - Favorable Intermediate-Risk Prostate Cancer

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

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

Thyroid Cancer or Indeterminate *Thyroid Nodule Testing*

The use of GEP testing for ~~Molecular profiling of~~ thyroid nodules with indeterminate cytology (e.g., Afirma® Genomic Sequencing Classifier [~~-~~GSC]~~,~~ ThyroSeq® V3, ThyGeNEXT®~~/~~ThyraMIR®)~~, or the gene and gene fusion panel *BRAF, RAS, HRAS, NRAS, RET/PTC1, RET/PTC3, PAX8/PPARγ*~~ is proven and medically necessary when all of the following criteria are met:

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

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

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

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

~~Molecular CGP profiling~~ of confirmed anaplastic thyroid cancer ~~thyroid cancer (except anaplastic thyroid cancer) with genes thyroid nodules or gene panels (*NTRK, ALK, MMR, MSI, RAS, HRAS, NRAS, RET/PTC1, RET/PTC3, PAX8/PPARγ*) thyroid cancers~~ is ~~un~~proven and ~~not~~ medically necessary ~~for all other indications due to insufficient evidence of efficacy~~.
For all other primary thyroid cancers see criteria for FoundationOne® CDx below.

~~*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.*~~

Uveal Melanoma Gene Expression Profiling (GEP)

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

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

Companion Diagnostics via Tissue Sample for Solid Tumor Cancers

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

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

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

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

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

Companion Diagnostics via Plasma Sample/Liquid Biopsy (cell-free DNA [cfDNA] or circulating tumor DNA [ctDNA]) for Solid Tumor Cancers

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

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

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

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

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

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

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

Hematological Cancer Testing

~~Molecular profiling using Chromosomal Microarray Analysis~~**analysis** ~~(e.g., Oncoscan, Reveal SNP Oncology, CGH or SNP array) is proven and medically necessary for individuals with acute leukemia.~~

Testing at initial diagnosis

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

- **Acute lymphoblastic leukemia**
- ~~Multiple myeloma~~
-

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

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

Measurable Residual Disease (MRD) testing after treatment

~~**Use of a Next Generation Sequencing profile test to assess minimal residual disease**~~**MRD testing (e.g., ClonoSeq® MRD~~**, MyMRD**~~) is proven and medically necessary when ordered by a hematologist or oncologist for individuals with all of the following**~~multiple myeloma when the following criteria are met~~**:**

- ~~Individual has acute myeloid leukemia (AML), or a~~**Acute lymphoblastic** leukemia ~~(ALL)~~ **or multiple myeloma; and**
- **T**~~t~~**esting** ~~is being performed within 3 months of~~ **occurs after 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~~~~had~~ ~~an allogenic or autologous bone marrow transplant; and there is no clinical evidence of disease for multiple myeloma;~~ ~~**and**~~

Companion Diagnostics for Hematological Cancers

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

- ~~Within 3 months of completing a treatment;~~ ~~**and**~~
- ~~Has no evidence of progression~~

~~**All other multigene, gene expression or microarray molecular profiling**~~ **CGP (e.g., FoundationOne Heme) 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~~

~~Liquid Uveal Melanoma~~

~~**Gene expression profile testing (e.g., DecisionDx-UM) is considered** **biopsy (circulating tumor cell free DNA) molecular profiling tests for non-small cell lung cancer are proven and medically necessary when used to assist with predicting disease severity and making treatment decisions in the following situations** **criteria is met:**~~

- ~~Individual The test selected has primary, localized uveal melanoma no more than 50 genes; **and**~~
- ~~No prior molecular profiling has been performed on the same tumor; and~~
- ~~There The individual is no evidence of metastatic disease 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~~
- ~~Has not previously had DecisionDx-UM testing for current diagnosis~~
- ~~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~~

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

Due to insufficient evidence of efficacy, molecular testing such as GEP ~~**profiling using gene expression profiling, Chromosome Microarray Analysis and**~~ **multi-gene NGS** ~~**cancer**~~ **panels and CGP** ~~**are**~~ **is unproven and not medically necessary for all** ~~**all other**~~ **indications other than those previously described as proven, including but not limited to:**

- **NGS panels of > 50 genes unless otherwise specified**
- ~~Bladder Cancer~~ ~~**cancer**~~ ~~(e.g., Decipher® Bladder) (NCCN, Bladder 2019a)~~
- ~~Cancers of unknown primary site (e.g., Response Dx~~ **Tissue of Origin™,** ~~CancerTYPE ID®, Rosetta Cancer Origin™, ProOnc, SourceDX, Pathfinder TG)~~
- ~~Pancreatic Cancer (e.g.,~~ **PancraGenEN®, PancreaSeq®**
- ~~)~~
- ~~Colorectal Cancer~~ ~~**cancer**~~ ~~(e.g., Oncotype DX®~~ ~~**Colon**~~ ~~Colon Cancer~~ ~~**cancer Assay assay**~~ ~~Assay~~, Colorectal Cancer DSA™, **Genefx℠,** ~~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®** ~~Decision Dx Melanoma, Decision Dx-UM,~~ DermTech **PLA™, myPath®-Melanoma** ~~PLA~~)
- ~~Multiple myeloma (e.g., MyPRS®/MyPRS Plus™)~~
- **Multi-cancer early detection/screening tests (e.g., Galleri®)**
- ~~Prostate cancer (e.g., Oncotype DX Prostate Cancer Assay,~~ TMPRSS2 fusion gene, Prolaris® Prostate Cancer Test, ~~Decipher Prostate Cancer Classifier,~~ **ExoDX Prostate** ~~IntelliScore [EPI]~~**Test, MiPS (Mi Prostate Score Urine test), MyProstateScore (MPS, formerly MiPS), Confirm MDx, Select MDx** ~~Classifer)~~
- **Tumor-informed assays (e.g., Invitae Personalized Cancer Monitoring, Signatera™, RaDaR®)** ~~)~~

- ~~Uveal melanoma (e.g., Decision Dx-UM)~~
- ~~Whole Exome Sequencing (WES) and Whole Genome~~~~Genomic~~ ~~Sequencing (WGS) of tumors~~ **MRD monitoring for solid tumors (e.g., Guardant Reveal™)**
- **Percepta® GSC for suspicious lung nodules**
- **Solid tumor profiling that includes Whole Exome, Whole Genome or whole transcriptome Sequencing (e.g., Caris MI Tumor Seek™, Caris MI Profile™, Tempus xE)**
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Definitions

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

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

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

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

~~All platforms share in common 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).~~

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

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

Low-Risk Prostate Cancer: Clinical/pathological features must include all of the following: PSA less than 10, Gleason score ≤3 + 3, no more than 3 biopsy cores positive

with no more than 50% cancer in each core and palpable disease (T1-T2a) (NCCN Prostate Cancer, v1.2023).

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

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

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

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

Whole Exome Sequencing (WES): About 1% of a person's DNA makes protein. These protein-making sections are called exons. All the exons together are called the exome. WES is a DNA analysis technique that looks at all ~~of~~ 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~~**MedlinePlus, 2020**).

Whole Genome Sequencing (WGS): WGS determines the sequence of the entire DNA in a person, or a tissue type, such as a tumor, which includes the protein-making (coding) as well as non-coding DNA elements (~~U.S. National Library of Medicine, 2017B~~**MedlinePlus, 2020**).

Applicable Codes

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

CPT Code	Description
***0005U**	**Oncology (prostate) gene expression profile by real-time RT-PCR of 3 genes (ERG, PCA3, and SPDEF), urine, algorithm reported as risk score**
***0011M**	Oncology, prostate cancer, mRNA expression assay of 12 genes (10 content and 2 housekeeping), RT-PCR test utilizing blood plasma and urine, algorithms to predict high-grade prostate cancer risk
***0012M**	Oncology (urothelial), mRNA, gene expression profiling by real-time quantitative PCR of five genes (MDK, HOXA13, CDC2 [CDK1], IGFBP5, and CXCR2), utilizing urine, algorithm reported as a risk score for having urothelial carcinoma

CPT Code	Description
*0013M	Oncology (urothelial), mRNA, gene expression profiling by real-time quantitative PCR of five genes (MDK, HOXA13, CDC2 [CDK1], IGFBP5, and CXCR2), utilizing urine, algorithm reported as a risk score for having recurrent urothelial carcinoma
***0016M**	**Oncology (bladder), mRNA, microarray gene expression profiling of 219 genes, utilizing formalin-fixed paraffin-embedded tissue, algorithm reported as molecular subtype (luminal, luminal infiltrated, basal, basal claudin-low, neuroendocrine-like)**
***0017M**	**Oncology (diffuse large B-cell lymphoma [DLBCL]), mRNA, gene expression profiling by fluorescent probe hybridization of 20 genes, formalin-fixed paraffin-embedded tissue, algorithm reported as cell of origin**
~~0005U~~	~~Oncology (prostate) gene expression profile by real-time RT-PCR of 3 genes (ERG, PCA3, and SPDEF), urine, algorithm reported as risk score~~
*0018U	Oncology (thyroid), microRNA profiling by RT-PCR of 10 microRNA sequences, utilizing fine needle aspirate, algorithm reported as a positive or negative result for moderate to high risk of malignancy
*0019U	Oncology, RNA, gene expression by whole transcriptome sequencing, formalin-fixed paraffin embedded tissue or fresh frozen tissue, predictive algorithm reported as potential targets for therapeutic agents
*0021U	Oncology (prostate), detection of 8 autoantibodies (ARF 6, NKX3-1, 5'-UTR-BMI1, CEP 164, 3'-UTR-Ropporin, Desmocollin, AURKAIP-1, CSNK2A2), multiplexed immunoassay and flow cytometry serum, algorithm reported as risk score
*0022U	Targeted genomic sequence analysis panel, 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
*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

CPT Code	Description
*0048U	Oncology (solid organ neoplasia), DNA, targeted sequencing of protein-coding exons of 468 cancer-associated genes, including interrogation for somatic mutations and microsatellite instability, matched with normal specimens, utilizing formalin-fixed paraffin-embedded tumor tissue, report of clinically significant mutation(s)
*0050U	Targeted genomic sequence analysis panel, acute myelogenous leukemia, DNA analysis, 194 genes, interrogation for sequence variants, copy number variants or rearrangements
*0069U	Oncology (colorectal), microRNA, RT-PCR expression profiling of miR-31-3p, formalin-fixed paraffin-embedded tissue, algorithm reported as an expression score
~~0081U~~	~~Oncology (uveal melanoma), mRNA, gene expression profiling by real-time RT-PCR of 15 genes (12 content and 3 housekeeping genes), utilizing fine needle aspirate or formalin-fixed paraffin-embedded tissue, algorithm reported as risk of metastasis~~
*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
***0120U**	**Oncology (B-cell lymphoma classification), mRNA, gene expression profiling by fluorescent probe hybridization of 58 genes (45 content and 13 housekeeping genes), formalin-fixed paraffin-embedded tissue, algorithm reported as likelihood for primary mediastinal B-cell lymphoma (PMBCL) and diffuse large B-cell lymphoma (DLBCL) with cell of origin subtyping in the latter**
*0153U	Oncology (breast), mRNA, gene expression profiling by next-generation sequencing of 101 genes, utilizing formalin-fixed paraffin-embedded tissue, algorithm reported as a triple negative breast cancer clinical subtype(s) with information on immune cell involvement
*0171U	Targeted genomic sequence analysis panel, acute myeloid leukemia, myelodysplastic syndrome, and myeloproliferative neoplasms, DNA analysis, 23 genes, interrogation for sequence variants, rearrangements and minimal residual disease, reported as presence/absence
***0179U**	**Oncology (non-small cell lung cancer), cell-free DNA, targeted sequence analysis of 23 genes (single nucleotide variations, insertions and deletions, fusions without prior knowledge of partner/breakpoint, copy number variations), with report of significant mutation(s)**

CPT Code	Description
*0204U	Oncology (thyroid), mRNA, gene expression analysis of 593 genes (including BRAF, RAS, RET, PAX8, and NTRK) for sequence variants and rearrangements, utilizing fine needle aspirate, reported as detected or not detected
*0211U	Oncology (pan-tumor), DNA and RNA by next-generation sequencing, utilizing formalin-fixed paraffin-embedded tissue, interpretative report for single nucleotide variants, copy number alterations, tumor mutational burden, and microsatellite instability, with therapy association
*0239U	Targeted genomic sequence analysis panel, solid organ neoplasm, cell-free DNA, analysis of 311 or more genes, interrogation for sequence variants, including substitutions, insertions, deletions, select rearrangements, and copy number variations
*0242U	Targeted genomic sequence analysis panel, solid organ neoplasm, cell-free circulating DNA analysis of 55-74 genes, interrogation for sequence variants, gene copy number amplifications, and gene rearrangements
*0244U	Oncology (solid organ), DNA, comprehensive genomic profiling, 257 genes, interrogation for single-nucleotide variants, insertions/deletions, copy number alterations, gene rearrangements, tumor-mutational burden and microsatellite instability, utilizing formalin-fixed paraffin-embedded tumor tissue
*0245U	Oncology (thyroid), mutation analysis of 10 genes and 37 RNA fusions and expression of 4 mRNA markers using next-generation sequencing, fine needle aspirate, report includes associated risk of malignancy expressed as a percentage
*0250U	Oncology (solid organ neoplasm), targeted genomic sequence DNA analysis of 505 genes, interrogation for somatic alterations (SNVs [single nucleotide variant], small insertions and deletions, one amplification, and four translocations), microsatellite instability and tumor-mutation burden
*0262U	Oncology (solid tumor), gene expression profiling by real-time RT-PCR of 7 gene pathways (ER, AR, PI3K, MAPK, HH, TGFB, Notch), formalin-fixed paraffin-embedded (FFPE), algorithm reported as gene pathway activity score
*0285U	Oncology, response to radiation, cell-free DNA, quantitative branched chain DNA amplification, plasma, reported as a radiation toxicity score
*0287U	Oncology (thyroid), DNA and mRNA, next-generation sequencing analysis of 112 genes, fine needle aspirate or formalin-fixed paraffin-embedded (FFPE) tissue, algorithmic prediction of cancer recurrence, reported as a categorical risk result (low, intermediate, high)
*0288U	Oncology (lung), mRNA, quantitative PCR analysis of 11 genes (BAG1, BRCA1, CDC6, CDK2AP1, ERBB3, FUT3, IL11, LCK, RND3, SH3BGR, WNT3A) and 3 reference genes (ESD, TBP, YAP1), formalin-fixed paraffin-embedded (FFPE) tumor tissue, algorithmic interpretation reported as a recurrence risk score

CPT Code	Description
*0296U	Oncology (oral and/or oropharyngeal cancer), gene expression profiling by RNA sequencing at least 20 molecular features (efeaturese.g.,.eg, human and/or microbial mRNA), saliva, algorithm reported as positive or negative for signature associated with malignancy
*0297U	Oncology (pan tumor), whole genome sequencing of paired malignant and normal DNA specimens, fresh or formalin-fixed paraffin-embedded (FFPE) tissue, blood or bone marrow, comparative sequence analyses and variant identification
*0298U	Oncology (pan tumor), whole transcriptome sequencing of paired malignant and normal RNA specimens, fresh or formalin-fixed paraffin-embedded (FFPE) tissue, blood or bone marrow, comparative sequence analyses and expression level and chimeric transcript identification..
*0299U	Oncology (pan tumor), whole genome optical genome mapping of paired malignant and normal DNA specimens, fresh frozen tissue, blood, or bone marrow, comparative structural variant identification
*0300U	Oncology (pan tumor), whole genome sequencing and optical genome mapping of paired malignant and normal DNA specimens, fresh tissue, blood, or bone marrow, comparative sequence analyses and variant identification
*0306U	Oncology (minimal residual disease [MRD]), next-generation targeted sequencing analysis, cell-free DNA, initial (baseline) assessment to determine a patient specific panel for future comparisons to evaluate for MRD
*0307U	Oncology (minimal residual disease [MRD]), next-generation targeted sequencing analysis of a patient-specific panel, cell-free DNA, subsequent assessment with comparison to previously analyzed patient specimens to evaluate for MRD
*0313U	Oncology (pancreas), DNA and mRNA next-generation sequencing analysis of 74 genes and analysis of CEA (CEACAM5) gene expression, pancreatic cyst fluid, algorithm reported as a categorical result (i.e.,ie, negative, low probability of neoplasia or positive, high probability of neoplasia)
*0314U	Oncology (cutaneous melanoma), mRNA gene expression profiling by RT-PCR of 35 genes (32 content and 3 housekeeping), utilizing formalin-fixed paraffin-embedded (FFPE) tissue, algorithm reported as a categorical result (ie, benign, intermediate, malignant)
*0315U	Oncology (cutaneous squamous cell carcinoma), mRNA gene expression profiling by RT-PCR of 40 genes (34 content and 6 housekeeping), utilizing formalin-fixed paraffin-embedded (FFPE) tissue, algorithm reported as a categorical risk result (ie, Class 1, Class 2A, Class 2B)
*0326U	Targeted genomic sequence analysis panel, solid organ neoplasm, cell-free circulating DNA analysis of 83 or more genes, interrogation for sequence variants, gene copy number amplifications, gene rearrangements, microsatellite instability and tumor mutational burden
*0329U	Oncology (neoplasia), exome and transcriptome sequence analysis for sequence variants, gene copy number amplifications and deletions, gene rearrangements, microsatellite instability and tumor mutational burden utilizing DNA and RNA from tumor with DNA from normal blood or saliva for subtraction, report of clinically significant mutation(s) with therapy associations

CPT Code	Description
*0331U	Oncology (hematolymphoid neoplasia), optical genome mapping for copy number alterations and gene rearrangements utilizing DNA from blood or bone marrow, report of clinically significant alterations
*0332U	Oncology (pan-tumor), genetic profiling of 8 DNA-regulatory (epigenetic) markers by quantitative polymerase chain reaction (qPCR), whole blood, reported as a high or low probability of responding to immune checkpoint-inhibitor therapy
*0333U	Oncology (liver), surveillance for hepatocellular carcinoma (HCC) in high-risk patients, analysis of methylation patterns on circulating cell-free DNA (cfDNA) plus measurement of serum of AFP/AFP-L3 and oncoprotein des-gamma-carboxy-prothrombin (DCP), algorithm reported as normal or abnormal result
*0334U	Oncology (solid organ), targeted genomic sequence analysis, formalin-fixed paraffin-embedded (FFPE) tumor tissue, DNA analysis, 84 or more genes, interrogation for sequence variants, gene copy number amplifications, gene rearrangements, microsatellite instability and tumor mutational burden
*0339U	Oncology (prostate), mRNA expression profiling of HOXC6 and DLX1, reverse transcription polymerase chain reaction (RT-PCR), first-void urine following digital rectal examination, algorithm reported as probability of high-grade cancer
*0340U	Oncology (pan-cancer), analysis of minimal residual disease (MRD) from plasma, with assays personalized to each patient based on prior next-generation sequencing of the patient's tumor and germline DNA, reported as absence or presence of MRD, with disease-burden correlation, if appropriate
*0343U	Oncology (prostate), exosome-based analysis of 442 small noncoding RNAs (sncRNAs) by quantitative reverse transcription polymerase chain reaction (RT-qPCR), urine, reported as molecular evidence of no-, low-, intermediate- or high-risk of prostate cancer
***0356U**	**Oncology (oropharyngeal), evaluation of 17 DNA biomarkers using droplet digital PCR (ddPCR), cell-free DNA, algorithm reported as a prognostic risk score for cancer recurrence**
***0362U**	**Oncology (papillary thyroid cancer), gene-expression profiling via targeted hybrid capture-enrichment RNA sequencing of 82 content genes and 10 housekeeping genes, formalin-fixed paraffin embedded (FFPE) tissue, algorithm reported as one of three molecular subtypes**
***0363U**	**Oncology (urothelial), mRNA, gene ~~[1]~~expression profiling by real-time quantitative PCR of 5 genes (MDK, HOXA13, CDC2 [CDK1], IGFBP5, and CXCR2), utilizing urine, algorithm incorporates age, sex, smoking history, and macrohematuria frequency, reported as a risk score for having urothelial carcinoma**
*81228	Cytogenomic (genome-wide) analysis for constitutional chromosomal abnormalities; interrogation of genomic regions for copy number variants, comparative genomic hybridization [CGH] microarray analysis

CPT Code	Description
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, 5-50 genes (eg, ALK, BRAF, CDKN2A, EGFR, ERBB2, KIT, KRAS, MET, NRAS, PDGFRA, PDGFRB, PGR, PIK3CA, PTEN, RET), interrogation for sequence variants and copy number variants or rearrangements, if performed; DNA analysis or combined DNA and RNA analysis** ~~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~~**analysis**
***81449**	**Targeted genomic sequence analysis panel, solid organ neoplasm, 5-50 genes (eg, ALK, BRAF, CDKN2A, EGFR, ERBB2, KIT, KRAS, MET, NRAS, PDGFRA, PDGFRB, PGR, PIK3CA, PTEN, RET), interrogation for sequence variants and copy number variants or rearrangements, if performed; RNA analysis**
*81450	**Targeted genomic sequence analysis panel, hematolymphoid neoplasm or disorder, 5-50 genes (eg, BRAF, CEBPA, DNMT3A, EZH2, FLT3, IDH1, IDH2, JAK2, KIT, KRAS, MLL, NOTCH1, NPM1, NRAS), interrogation for sequence variants, and copy number variants or rearrangements, or isoform expression or mRNA expression levels, if performed; DNA analysis or combined DNA and RNA analysis** ~~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~~**analysis**
***81451**	**Targeted genomic sequence analysis panel, hematolymphoid neoplasm or disorder, 5-50 genes (eg, BRAF, CEBPA, DNMT3A, EZH2, FLT3, IDH1, IDH2, JAK2, KIT, KRAS, MLL, NOTCH1, NPM1, NRAS), interrogation for sequence variants, and copy number variants or rearrangements, or isoform expression or mRNA expression levels, if performed; RNA analysis**

CPT Code	Description
*81455	**Targeted genomic sequence analysis panel, solid organ or hematolymphoid neoplasm or disorder, 51 or greater genes (eg, ALK, BRAF, CDKN2A, CEBPA, DNMT3A, EGFR, ERBB2, EZH2, FLT3, IDH1, IDH2, JAK2, KIT, KRAS, MET, MLL, NOTCH1, NPM1, NRAS, PDGFRA, PDGFRB, PGR, PIK3CA, PTEN, RET), interrogation for sequence variants and copy number variants or rearrangements, or isoform expression or mRNA expression levels, if performed; DNA analysis or combined DNA and RNA analysis** ~~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~~~~analysis~~
***81456**	**Targeted genomic sequence analysis panel, solid organ or hematolymphoid neoplasm or disorder, 51 or greater genes (eg, ALK, BRAF, CDKN2A, CEBPA, DNMT3A, EGFR, ERBB2, EZH2, FLT3, IDH1, IDH2, JAK2, KIT, KRAS, MET, MLL, NOTCH1, NPM1, NRAS, PDGFRA, PDGFRB, PGR, PIK3CA, PTEN, RET), interrogation for sequence variants and copy number variants or rearrangements, or isoform expression or mRNA expression levels, if performed; RNA analysis**
81479	Unlisted molecular pathology procedure
*81504	Oncology (tissue of origin), microarray gene expression profiling of > 2000 genes, utilizing formalin-fixed paraffin-embedded tissue, algorithm reported as tissue similarity scores
*81518	Oncology (breast), mRNA, gene expression profiling by real-time RT-PCR of 11 genes (7 content and 4 housekeeping), utilizing formalin-fixed paraffin-embedded tissue, algorithms reported as percentage risk for metastatic recurrence and likelihood of benefit from extended endocrine therapy
81519	Oncology (breast), mRNA, gene expression profiling by real-time RT-PCR of 21 genes, utilizing formalin-fixed paraffin embedded tissue, algorithm reported as recurrence score
*81520	Oncology (breast), mRNA gene expression profiling by hybrid capture of 58 genes (50 content and 8 housekeeping), utilizing formalin-fixed paraffin-embedded tissue, algorithm reported as a recurrence risk score
*81521	Oncology (breast), mRNA, microarray gene expression profiling of 70 content genes and 465 housekeeping genes, utilizing fresh frozen or formalin-fixed paraffin-embedded tissue, algorithm reported as index related to risk of distant metastasis
*81522	Oncology (breast), mRNA, gene expression profiling by RT-PCR of 12 genes (8 content and 4 housekeeping), utilizing formalin-fixed paraffin-embedded tissue, algorithm reported as recurrence risk score
*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

CPT Code	Description
*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 ~~81545~~	Oncology (thyroid), **mRNA,** gene expression analysis of **10,196** ~~142~~ genes, utilizing fine needle aspirate, algorithm reported as a categorical result (e.g., benign or suspicious)
*81551	Oncology (prostate), promoter methylation profiling by real-time PCR of 3 genes (GSTP1, APC, RASSF1), utilizing formalin-fixed paraffin-embedded tissue, algorithm reported as a likelihood of prostate cancer detection on repeat biopsy
*81552	Oncology (uveal melanoma), mRNA, gene expression profiling by real-time RT-PCR of 15 genes (12 content and 3 housekeeping), utilizing fine needle aspirate or formalin-fixed paraffin-embedded tissue, algorithm reported as risk of metastasis
*81599	Unlisted multianalyte assay with algorithmic analysis
*86152	Cell enumeration using immunologic selection and identification in fluid specimen (e.g., circulating tumor cells in blood**);**~~)~~
*86153	Cell enumeration using immunologic selection and identification in fluid specimen (e.g., circulating tumor cells in blood); physician interpretation and report, when required

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

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

Diagnosis Code	Description
C90.10	Plasma cell leukemia not having achieved remission

Diagnosis Code	Description
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
~~C91.40~~	~~Hairy cell leukemia not having achieved remission~~
~~C91.41~~	~~Hairy cell leukemia, in remission~~
~~C91.42~~	~~Hairy cell leukemia, in relapse~~
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 **Analysis** and Next Generation Sequencing, as well as others that assess the gene products, such as gene expression arrays and microRNA analysis. The number of genes evaluated can range from a single gene to the whole exome or genome of a tumor. For the purposes of this policy, multi-gene analysis generally refers to a gene panel containing five or more genes, though some exceptions may apply as noted specifically in the policy ~~(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

Thyroid Cancer/Indeterminate Thyroid Nodules

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

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

In 2022, Babazadeh et al. reported on the clinical utility of Afirma XA testing during two years of clinical use. Afirma XA became available in 2018 and assesses 593 genes,

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

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

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

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

In a prospective blinded, multicenter study by Steward et al. (2019, included in the Lee et al. 2022 systematic review and the Hayes ThyroSeq v3 Genomic Classifier Molecular Test Assessment above), authors sought to find the diagnostic exactness of a multigene classifier test (ThyroSeq v3) for cytologically indeterminate thyroid nodules. The study enrolled 782 individuals with a total of 1,013 nodules. Of those, 286 FNA samples from 256 individuals met inclusion criteria and underwent molecular analysis with the multigene GC (ThyroSeq v3). The primary outcome of this study was the correct separation of benign histopathological nodules from cancer and noninvasive follicular thyroid neoplasms with papillary-like nuclei (NIFTP) in samples with Bethesda III and IV cytology. Of the 286 cytologically indeterminate nodules, 206 (72%) were benign, 69 (24%) were malignant, and 11 (4%) were noninvasive follicular thyroid neoplasms with papillary-like nuclei (NIFTP). Overall, 257 (90%) nodules (154 Bethesda III, 93 Bethesda IV, and 10

Bethesda V) had informative GC analysis, with 61% classified as negative and 39% as positive. The test collectively established a 94% (95% CI, 86%-98%) sensitivity and 82% (95% CI, 75%-87%) specificity in Bethesda III and IV nodules. With a cancer/NIFTP incidence of 28%, the negative predictive value (NPV) was 97% (95% CI, 93%-99%), and the PPV was 66% (95% CI, 56%-75%). The detected 3% false-negative rate was comparable to benign cytology; the missed cancers were all low-risk tumors. Between nodules testing positive, precise groups of genetic variations had cancer likelihoods fluctuating from 59% to 100%. Limitations to the study include a small sample size and no long-term clinical impact outcomes established. The authors concluded the multigene genomic classifier test (ThyroSeq v3) showed high sensitivity/NPV and relatively high specificity/PPV, which could eliminate the need for diagnostic surgical procedures in up to 82% of all benign thyroid nodules with indeterminate cytology and 61% of individuals with Bethesda III to IV indeterminate nodules.

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

~~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, individuals** ~~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%.~~

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~~ individuals with primary thyroid medullary cancer.

- **Post-operative radioiodine (RAI) therapy. Molecular testing to guide postoperative RAI use is not recommended at this time.**

American Association of Endocrine Surgeons (AAES)

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

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

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

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

~~The~~ National Comprehensive Cancer Network (NCCN)

The 2022 NCCN guidelines ~~on~~for t~~T~~hyroid ~~C~~carcinoma~~ancer~~ indicate~~s~~ that molecular diagnostics may be helpful to reclassify follicular lesions, based on genetic profile, as more /less likely to be benign or malignant. ~~for thyroid nodules with indeterminate cytology or to reclassify follicular lesions.~~ In addition, molecular testing specific to medullary thyroid cancer in Bethesda lll-Vl nodules may identify these unique carcinoma types, as it is challenging to explicitly identify them via cytology (category 2B evidence). ~~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~~Although past studies have shown that molecular diagnostics do not perform well for Hürthle cell

neoplasms, modern genomic classifiers are promising with regard to Hürthle cell specimens. A requirement for the diagnosis of Hürthle cell and follicular carcinomas is evidence of either vascular or capsular invasion, which fine needle aspiration cannot determine; use of molecular diagnostics may be considered in these situations. ~~They note that m~~**Molecular markers, however, should be interpreted with caution and used in conjunction with individualized clinical, radiographic and cytologic features. The NCCN panel notes that molecular testing has been shown to have benefit for making targeted treatment decisions, especially those related to use of drug therapy or clinical trial participation. Some mutations may also have prognostic importance. Molecular testing of single genes or a gene expression classifier panel test may be** ~~considered, and~~**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.~~**(NCCN Thyroid Carcinoma, v3.2022)**

Hematological Malignancies

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

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

Evans et al. (2016) studied the diagnostic utility of SNP + CGH array to identify unexplained cytopenia in 83 MDS patients and compared results with 18 normal bone marrow controls. Array analysis was done in parallel with standard cytogenetics, FISH, flow

cytometry, and morphology. Forty-five percent of patients were diagnosed with MDS, 33% were normal, and 8% had other pathological disorders. 57% of the MDS patients had normal cytogenetics, but the SNP + CGH array found significant cryptic chromosome aberrations. In MDS patients with abnormal cytogenetics, the array essentially matched the chromosome results and didn't add any new information. Overall, the SNP + CGH array analysis contributed significantly to the diagnostic yield in indeterminate morphology cytopenic patients.

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

~~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. **(201~~5~~4)** 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 Measurable~~Minimal~~ Residual Disease (MRD) in Hematologic Malignancies

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

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

relapse. In this systematic review, using a threshold of 0.1%, 5 year DFT of 63% indicates that a significant portion of MRD-negative individuals will still relapse. In opposition, a small percentage (16%) of individuals who were MRD-positive were still disease free at 5 years. Overall, the authors concluded that for individuals with AML in remission, MRD-negativity correlates with higher DFS and OS, which provides further support for the use of MRD in individuals with AML.

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

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

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

The efficacy of targeted NGS to identify MRD in patients with acute myeloid leukemia (AML) was studied by Jongen-Lavrencic et al. (2018). Between 2001 and 2013, a total of 482 patients ranging in age from 18-65 with newly diagnosed AML were included. NGS of 54 genes that are often present in AML patients was performed at diagnosis and after induction therapy during complete remission. The end points analyzed were 4-year relapse, relapse free survival and overall survival. Results were compared with flow cytometry (FC). The authors discovered an average of 2.9 mutations per patient, of which at least one single mutation could serve as an indicator of residual disease, in 430 patients. These patients then had NGS testing repeated on bone marrow after induction therapy**, and**

they were in complete remission. Persistent mutations were found in 52% and were highly variable across the genes analyzed. DTA mutations were most common, persisting at rates of 79%, whereas *RAS* pathway mutations cleared, persisting at an average rate of about 9%. The authors noted that DTA mutations are common gene mutations in individuals with age related clonal hematopoiesis, and likely represent non-leukemic clones rather than persistent malignant disease. After DTA mutations were excluded, the detection of MRD was associated with a significantly higher relapse rate than no detection (55% vs. 32%), lower relapse-free survival (37% vs. 58%) and overall survival (42% vs. 66%). The results of NGS were compared to FC in a subset of 340 patients. Concordant results for detection or non-detection of MRD were found in 69% of patients. **The four-year relapse rate was 73% among patients in whom both assays were positive, 52% among those who had residual disease on sequencing but not on flow cytometry, 49% among those who had residual disease on flow cytometry but not on sequencing, and 27% among those in whom both assays were negative. Multivariate analysis found that combining the two assays gave a high prognostic value to the rate of relapse (p <.**001), relapse free survival (p < .001) and overall survival (p = .003).The authors concluded that persistent mutations associated with clonal hematopoiesis did not have prognostic value, whereas the detection of MRD during complete remission using NGS with FC had significant additive prognostic value.

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

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

Avet-Loiseau et al. **(2015) reported on the use of FC and NGS in the Intergroup** Francophone du Myélome/ Dana-Farber Cancer Institute (IFM/DFCI) 2009 trial to measure MRD in the IFM arm of the study. This trial enrolled 700 patients under 66 years of age and randomized them to either receive either 8 cycles of VRD (Velcade-Revlimid-Dexamethasone) (arm A), or 3 VRD cycles, high-dose melphalan, followed by two consolidation VRD cycles (arm B). All patients received a lenalidomide maintenance for 12 months. A total of 246 patients were evaluated by NGS using the LymphoSight platform, and before maintenance, 87 patients were negative, 80 were low-positive, and 79 were positive. After maintenance, 178 were tested, and 86 patients were negative, 52 were low-positive, and 40 were positive. Using a cutoff of 10^{-6}, patients below this threshold had a pre-maintenance

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

~~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.~~

Clinical Practice Guidelines

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

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

- **There is currently insufficient evidence to make modifications to maintenance therapy based on depth of response, including MRD status (Type: informal consensus/evidence**

based; Evidence quality: low/intermediate, benefit outweighs harm; Strength of recommendation: moderate).

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

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

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

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

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

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

European Society for Medical Oncology (ESMO)

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

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

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

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

National Comprehensive Cancer Network (NCCN)

NCCN guidelines for ALL (v1.2022) recommend molecular characterization using FISH testing, reverse transcriptase-polymerase chain reaction testing and comprehensive testing via NGS for gene fusions and pathogenic mutations. Optional tests include CMA in cases of aneuploidy or failed karyotype. Regarding MRD, NCCN recommends ~~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~~.~~ **(NCCN Pediatric Acute Lymphoblastic Leukemia, v1.2023).**

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The NCCN guidelines for AML **indicate that multiplex gene panels and comprehensive NGS analysis are indicated for ongoing management of AML. Additionally, the guidelines** state that the role of MRD is evolving in both prognosis and treatment and that clinical trial participation is encouraged ~~(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 **(NCCN Acute Myeloid Leukemia, v2.2022).**

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

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

NCCN clinical practice guidelines for multiple myeloma state that single nucleotide polymorphism array or next generation sequencing panels on bone marrow have the potential to provide further risk categorization which may add prognostic value. No patient selection criteria were provided. **The NCCN Multiple Myeloma Panel suggests baseline clone identification and consideration of MRD as indicated for prognostication** (NCCN~~,~~ Multiple Myeloma**, v2.**202~~2~~**3**).

~~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).~~

Lung Cancer Tissue Testing

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

A comparison study by Yao et al. (2021) was performed to develop a quick gene testing procedure using fresh core needle biopsy samples from NSCLC patients. Thirty patients with NSCLC confirmed by frozen section examination were enrolled to compare the results of multi-gene mutation testing using fresh frozen (FF) tissues and paired formalin-fixed paraffin-embedded (FFPE) tissues. A total of 77 fresh NSCLC tissue samples obtained from core needle biopsy were evaluated by frozen section examination. The 77 patients consisted of 39 males (50.6%) and 38 females (49.4%) with a median age of 65 years (range, 42-85 years) of which 32 were smokers (41.6%) vs. 45 nonsmokers (58.4%). Frozen section examination revealed 70 (90.9%) AC, 6 (7.8%) SCC, and 1 (1.3%) adenosquamous carcinoma (ASC), which is consistent with the final pathological diagnosis using FFPE tissues. If the NSCLC diagnosis and adequate tumor cell counts were confirmed by histopathology, the fresh tissues were used to extract DNA and subsequent gene testing by ARMS-PCR. The paired FFPE core needle biopsy samples were from 30 NSCLC patients in stage IV, randomly selected for this study, who also underwent gene testing. The 77 fresh samples showed an EGFR mutation rate of 61.0%. The clinical treatment strategy for patients was optimized based on gene test results. Using this procedure of gene mutation testing, the time interval between physicians requesting and obtaining a test result has been shortened to fewer than 2 days. Following a comparison of gene testing results with fresh tissues and paired FFPE tissues from the 30 patients, no difference in the DNA concentration extracted from fresh tissues and FFPE tissues was found. DNA purity, however, was higher in fresh tissues than that in FFPE tissues. Gene testing detected the same gene mutations in 93.3% of cases in fresh tissues and paired FFPE tissues. The authors concluded that gene testing procedure using fresh biopsy samples greatly shortens the waiting time of patients. The multi-gene mutation testing using fresh core needle biopsy samples from NSCLC patients is a reasonable, achievable, and quick approach. The

authors stated that fresh tissues may serve as a potential alternative to FFPE tissues for gene testing in NSCLC patients. Limitations to this study include a risk of misdiagnosis during frozen section examination and uncertain diagnosis of fresh tissues related to lack of pathologist experience. Additionally, the sensitivity and specificity of gene testing using FF tissues are 96 and 75% when compared with FFPE tissues. The high sensitivity and low specificity may be attributed to the selection of cases through frozen section examination. The sample size is too small to prove the usefulness of this test as a diagnostic tool. Further research with randomized controlled trials is needed to validate these findings.

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

Drilon et al. **(2015) identified 31 patients with lung adenocarcinoma with a ≤ 15 pack-year smoking history whose tumors previously tested "negative" for alterations in 11 genes (mutations in EGFR, ERBB2, KRAS, NRAS, BRAF, MAP2K1, PIK3CA, and AKT1 and fusions involving ALK, ROS1, and RET) via multiple non-NGS methods. A broad, hybrid capture-based NGS assay (Foundation One**) was performed (4,557 exons of 287 cancer-related genes and 47 introns of 19 genes frequently rearranged in solid tumors). A genomic alteration with a corresponding targeted therapeutic based on the National Comprehensive Cancer Network (NCCN) guidelines for non-small cell lung cancer (NSCLC) was found in 26% (n = 8 of 31) of patients. **The drivers identified in tumors from these 8** patients were EGFR G719A, BRAF V600E, SOCS5-ALK, HIP1-ALK, CD74-ROS1, KIF5B-RET (n = 2), and CCDC6-RET. Six of these patients went on to receive targeted therapy. The authors noted that the reasons for non-detection of these genomic alterations via non-NGS testing can be varied such as lower sensitivity, complex rearrangements undetectable by standard FISH, and, possibly, heterogeneity between different tumor biopsies or sites. They concluded that broad, hybrid capture-based NGS assays have the potential to uncover clinically actionable genomic alterations in never smokers or ≤ 15 pack-year smokers whose lung adenocarcinomas

do not harbor a potential driver via non-NGS testing. **(Oxnard et al., 2016, Riediger et al., 2016).**

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

Clinical Practice Guidelines

American College of Chest Physicians (ACCP)

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

American Society of Clinical Oncology (ASCO)

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

- Physicians may use molecular biomarker testing in tumors with:
  - An adenocarcinoma component;
  - Nonsquamous, non-small-cell histology;
  - Any non-small-cell histology when clinical features indicate a higher probability of an oncogenic driver (e.g., young age [< 50 years]; light or absent tobacco exposure).
- BRAF testing should be performed on all patients with advanced lung adenocarcinoma, irrespective of clinical characteristics. RET, or KRAS, or MET molecular testing are not recommended as single gene routine stand-alone assays outside the context of a clinical trial. It is appropriate to include these as part of larger testing panels performed either initially or when routine EGFR, ALK, BRAF, and ROS1 testing is negative.

- Multiplexed genetic sequencing panels are preferred where available over multiple single-gene tests to identify other treatment options beyond EGFR, ALK, BRAF, and ROS1.
- Circulating tumor cell free DNA testing, also called a liquid biopsy, should not be routinely considered due to lack of evidence of efficacy. However, the expert consensus opinion provided is that cfDNA may be used in some clinical settings in which tissue is limited and/or insufficient for molecular testing to identify EGFR mutations.

National Comprehensive Cancer Network (NCCN)

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

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

Melanoma

Cutaneous Melanoma

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

An Ontario Health Technology Assessment (2021) that evaluated the diagnostic accuracy, clinical utility and budget impact of pigmented lesion assays (PLA) for people with suspected melanoma skin lesions. The systematic review included seven studies consisting of six cohort studies (including three Ferris studies (2017, 2018 and 2019) that were previously discussed in this policy) and one survey that were conducted in dermatology

offices, examining adults (> 18 years old) with suspected melanoma lesions using the DermTech pigmented lesion assay. The authors stated that the risk of bias in the included studies was generally moderate to high, and the quality of evidence was very low. Limitations noted in the review included the potential bias from the industry sponsored studies, overestimation of the diagnostic accuracy of PLA, the diagnostic accuracy of visual assessment may have been underestimated when compared to published literature, and many parameters and assumptions used by the economic analysis were not reported in the study, which they stated had potentially serious limitations. They concluded that there was no evidence demonstrating the impact of PLA on patient outcomes and that the low-quality evidence for the diagnostic accuracy of PLA remains uncertain when compared to visual inspection alone. They also stated that the evidence is uncertain about whether PLA has an impact on clinical decision making and that the cost-effectiveness of this test compared with the standard care pathway is also uncertain.

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

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

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

~~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.~~

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

Zager et al. (2018, included in Hayes DecisionDx-Melanoma Molecular Test Assessment, above ~~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 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** ~~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~~~~the vast majority 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 in order to differentiate between melanoma and Spitz nevi. Sixteen patients with histologically ambivalent melanocytes were evaluated in the study, and of these, 8 haseight have positive sentinel lymph node biopsy, 1one of which also had distant metastasis. Also evaluated were 8eight patients with Spitz nevi, and 3three patients with melanoma (2two spitzoid, 1one superficial spreading). Chromosomal aberrations were found in 7seven 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 2two spitzoid melanomas, and 1one 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, 1one fatal metastatic case, and the other chromosomally aberrant ambivalent cases. It was positive in 1one spitzoid melanoma and 1one conventional. Overall, the authors concluded that CGH may offer better diagnostic aid with better sensitivity and specificity than FISH in atypical Spitz tumors.~~

~~NCCN (2019d) clinical practice guidelines for melanoma note that molecular profiling using a variety of tests ranging from cytogenetics to chromosome microarray to gene expression is more available to help stage indeterminate melanocytic neoplasms, at this time they offer only complementary information and their clinical utility is still under investigation. Gene expression tests are available that are being marketed to help determine prognosis, but it has not yet been established that these tests provide clinically actionable information beyond known nomograms using patient demographics and histopathology. The authors do note, however, that the analysis of some somatic genetic alterations such as *BRAF* and *KIT* may be useful to guide treatment decisions, but using whole genome or exome sequencing, or next generation sequencing panels to determine mutation burden, remain investigational.~~

Uveal Melanoma

Singh et al. (2022) conducted a retrospective 10-year cohort study to assess the accuracy of the predicted metastasis-free survival (MFS) rate by a gene expression profiling (GEP) test in patients with uveal melanoma (UM) by comparing the patients' GEP test results to

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

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

In a 5-year clinical outcome report from a prospective registry of individuals tested with a prognostic 15-gene expression profile (15-GEP) test for ~~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~~UM who underwent intraoperative fine needle

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

Plasseraud et al. (2016, **included in the Hayes DecisionDx-UM 2020 Molecular Test Assessment above**) evaluated the clinical validity and utility of DecisionDx-UM in a prospective, multicenter, study (supported by Castle Biosciences, Inc.). ~~70~~ Seventy patients were enrolled to document patient management differences and clinical outcomes associated with low-risk Class 1 and high-risk Class 2 results indicated by DecisionDx-UM testing. Thirty-seven patients in the prospective study were Class 1 and 33 were Class 2. Class 1 patients had 100% 3-year metastasis-free survival compared to 63% for Class 2 (log rank test p = 0.003) with 27.3 median follow-up months in this interim analysis. Class 2 patients received significantly higher-intensity monitoring and more oncology/clinical trial referrals compared to Class 1 patients (**Fisher's** ~~Fisher's~~ exact test p = 2.1 × 10(-13) and p = 0.04, resp.). In the authors' opinion, the results of this study provide additional, prospective evidence in an independent cohort of patients for which Class 1 and Class 2 patients are managed according to the differential metastatic risk indicated by DecisionDx-UM. A study limitation is financial sponsorship/support by the manufacturer which increases the risk of bias.

~~Mince 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.~~

Clinical Practice Guidelines

~~Professional Societies~~

American Academy of Dermatology (AAD)

Guidelines from the American Academy of Dermatology (~~The~~ AAD), updated in 2019, included recommendations for diagnostic, prognostic, and therapeutic ~~does not address~~ molecular testing **(Swetter et al., 2019).**

- **Ancillary diagnostic molecular techniques (e.g., comparative genomic hybridization; fluorescence** ~~in their guidelines at any point, and states~~ 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** ~~general that baseline laboratory tests~~ 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~~generally~~ not recommended in **the absence** ~~asymptomatic patients with newly diagnosed primary melanoma of any thickness, and that such tests have low yield for detection~~ of metastatic disease**.** ~~and are associated with relatively high false-positive rates (Bichakjian, 2011).~~

~~European Society for Medical Oncology (ESMO)~~

National Comprehensive Cancer Network (NCCN)

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

- **Prognostic gene expression profiling (GEP) to differentiate melanomas at low versus high risk for metastasis should not replace pathologic staging procedures, and the use of GEP testing according to specific AJCC-8 melanoma stage requires further prospective investigation in large, contemporary data sets of unselected patients.**
- ~~Available noninvasive pre-biopsy imaging and molecular technologies~~ **It remains unclear whether available GEP tests are reliably predictive of outcome across the risk spectrum as these tests have not been prospectively validated with clinical studies to accurately define the clinical utility of the tests.** ~~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 melanoma ~~at high risk for recurrence~~ for whom future BRAF-directed therapy may be an option.**

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

~~In their 2015 guidelines, ESMO states that genetic testing is generally not recommended for melanoma diagnoses, but notes that biomarkers such as mutations (NRAS, c-Kit, BRAF) are already indispensable today for a personalized medicine approach in advanced melanoma. Broader panels are not recommended, though it is noted that additional mutations and the overall mutation rate might provide additional molecular predictive markers in the near future (Drumer, 2015).~~

Cancers of Unknown Primary (CUP) ~~Site~~

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

Ross et al. (2021) performed a retrospective analysis of cancer of unknown primary (CUP) origin cases referred for comprehensive genomic profiling (CGP) to determine how many were potentially eligible for enrollment into an experimental CUPISCO arm, an ongoing

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

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

Therefore, for CUP patients it would be important to consider putative primary sites even when candidate actionable driver mutations are found. In addition, redundancy in activation of pathways of resistance does often take place as a mechanism of primary as well as secondary resistance. Further research is needed to determine the clinical relevance of these findings.

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

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

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

Clinical Practice Guidelines

~~Professional Societies~~

European Society for Medical Oncology (ESMO)

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

National Institute for Health and Care Excellence (NICE)

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

National Comprehensive Cancer Network (NCCN)

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

with the best individualized patient outcome in mind (NCCN Occult primary (Cancer of Unknown Primary [CUP]), v2.2023).

Colorectal Cancer (CRC)

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

Yothers et al. (2022) conducted a patient-specific meta-analysis of 12-gene colon cancer recurrence score validation studies for recurrence risk assessment after surgery with or without fluorouracil (5FU) and oxaliplatin. Three validation studies of the 12-gene colon recurrence score assay were used with pre-specified patient-specific meta-analysis (PSMA) methods to integrate the 12-gene Oncotype DX Colon Recurrence Score result (RS) with the clinical and pathology risk factors stage, T-stage, mis-match repair (MMR) status, and number of nodes examined to calculate individualized recurrence risk estimates. Baseline risk estimation used the most recent studies, so the risk estimates reflect current medical practice. The effect of 5FU was estimated with a meta-analysis of two studies. The effect of oxaliplatin was estimated using one of the RS assay validation studies, in which patients were randomized to 5FU with or without oxaliplatin. The RS result and each of the clinical-pathologic factors provided independent prognostic information for recurrence. Among stage II, T3, MMR-proficient patients with ≥12 nodes examined (the most common scenario), patients with RS ≤30 (approximately 48%) have estimated 5-year recurrence risk ≤10% with surgery alone. Among stage IIIA/B, T3, MMR-deficient patients with ≥12 nodes examined, patients with RS ≤19 (approximately 14%) have an estimated 5-year recurrence risk ≤10% with surgery alone. Among stage IIIA/B, T3, MMR-proficient patients with ≥12 nodes examined, those with RS ≤14 (approximately 6%) have estimated 5-year recurrence risk ≤10% with 5FU alone. The authors concluded that the PSMA integrates the 12-gene colon RS result with clinical and pathology factors to provide individualized recurrence risk estimates that reflect current medical practice. The risk estimates are in a range that may help inform treatment decisions for a substantial number of stage II and stage III patients. Limitations include that the estimated effect of 5FU is from a meta-analysis of a randomized study and a non-randomized treatment comparison with covariate adjustment to reduce bias. The SUNRISE study was a retrospective analysis that

selected patients who had not received adjuvant chemotherapy after resection for stage II or III colon cancer and this may have led to selection of patients whom clinicians had considered to be at lower risk of recurrence. Also, the PSMA risk assessment used a baseline risk assessment from the last two enrolling studies (NSABP C-07, enrolling from 2000-2002 and SUNRISE, enrolling from 2000-2005). If further improvements in patient outcomes have occurred since this time, they are not reflected in the present recurrence risk estimates. Finally, the RS result is not predictive, that is, it is not associated with the relative treatment effect of chemotherapy with 5FU or oxaliplatin. Further research with randomized controlled trials is needed to validate these findings.

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

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

Molecular technologies are also under investigation to screen for colon cancer, such as the Epi proColon 2.0 assay that measures the methylated Septin9 (SEPT9), a circulating tumor cell marker. The premise of this test is that during colorectal cancer development, the tumor will release cell free DNA (cfDNA) into the bloodstream, and the ratio of SEPT9 DNA be detected through specialized techniques **and can predict the presence of early colorectal cancer. A meta-analysis of one cohort study and thirteen case-controlled studies representing 9870 cases demonstrated a pooled sensitivity of 0.66 and specificity of 0.91.** The authors compared this to data available for the gold standard test, fecal

occult blood testing (FOBT) of a sensitivity of 0.60 and specificity of 0.91, equal to SEPT9. The authors combined the results of FOBT and SEPT9 and achieved a detection rate of colorectal cancer of 88.7% with a specificity of 78.8%. They concluded that FOBT and SEPT9 complement each other, but further studies are needed to determine the best screening tests and approaches (Yan et al., 2016).

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

To evaluate whether patients with CDX2-negative tumors might benefit from adjuvant chemotherapy, Dalerba et al. (2016) investigated the association between CDX2 status, and assessed at either the mRNA or protein level, the disease-free survival among patients who either did or did not receive adjuvant chemotherapy. Reviewing a database of 669 patients with stage II colon cancer and 1228~~1,228~~ patients with stage III colon cancer, the authors reported that their results confirmed that treatment with CDX2 as a biomarker in colon cancer adjuvant chemotherapy was associated with a higher rate of disease-free survival in both the stage II subgroup (91% with chemotherapy vs. 56% with no chemotherapy, p = 0.006) and the stage III subgroup (74% with chemotherapy vs. 37% with no chemotherapy, p < 0.001) of the CDX2-negative patient population (Fig. 5). A test for the interaction between the biomarker and the treatment revealed that the benefit observed in CDX2-negative cohorts was superior to that observed in CDX2-positive cohorts in both the stage II subgroup (p = 0.02 for the interaction) and the stage III subgroup (p = 0.005 for the interaction). In the authors' opinion, their results indicate that patients with stage II or stage III CDX2-negative colon cancer might benefit from adjuvant chemotherapy and that adjuvant chemotherapy might be a treatment option for patients with stage II CDX2-negative disease, who are commonly treated with surgery alone. Given the exploratory and retrospective design of this study, these results will need to be further validated through randomized, clinical trials, in conjunction with genomic DNA sequencing studies.

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

patients with stage IIIA to IIIB disease in the high-risk group had a recurrence risk similar to that of patients with stage IIIC disease in the low-risk group (approximately 38%). The authors conclude that this validation study of the 12-gene Recurrence Score assay in stage III colon cancer without chemotherapy showed the heterogeneity of recurrence risks in stage III as well as in stage II colon cancer.

~~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 cetuximab 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.~~

~~NCCN clinical practice guidelines for colon cancer review several multigene panels for prognosis and recurrence, including Oncotype Dx Colon, ColoPrint, and ColDx. The panels states that there is insufficient data to recommend the use of multigene assay panels to determine adjuvant therapy in colon cancer patients (NCCN, 2019c, 2019h).~~

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

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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 **was found**. Larger, prospective studies are needed to further confirm the performance of this test.

Clinical Practice Guidelines

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

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

National Comprehensive Cancer Network (NCCN)

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

Prostate Cancer

~~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 patient with higher grade disease and could reduce unnecessary biopsies; although 10% of prostate cancer cases would be missed based on the test characteristics.~~

~~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.~~

Decipher, Oncotype DX Prostate, Prolaris, and Promark

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

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

Feng et al. (2021) performed an ancillary study to validate the Decipher GC in men who received salvage radiation for elevated prostate-specific antigen (PSA) after surgery in the context of a phase 3 randomized trial. They used specimens from the placebo-controlled, phase 3 NRG/RTOG 9601 clinical trial and extracted RNA from the highest-grade tumor tissue available in 2019 (NRG/RTOG 9601 was conducted 1998-2003). Median follow up time was 13 years. GC scores were assigned (0-1) to whole transcriptomes and the predictive ability of GC for distant metastasis was evaluated. Additional outcomes including prostate cancer-specific mortality (PCSM) and overall survival (OS) were also

measured. The authors analyzed GC scores from 352 randomized participants who met quality-controlled inclusion criteria. The GC was found to have an association with distant metastasis (hazard ratio [HR], 1.17; 95% CI, 1.05-1.32; P = .006), PCSM (HR, 1.39; 95% CI, 1.20-1.63; P < .001) and OS (HR, 1.17; 95% CI, 1.06-1.29; P = .002) after adjusting for Gleason score, T stage, margin status, age, race/ethnicity, entry PSA and treatment arm, suggesting that not all men with biochemically recurrent cancer after surgical intervention will benefit equally from addition of hormone therapy to salvage radiotherapy. The researchers propose that the Decipher GC may hold promise for risk stratification and treatment decisions involving hormone therapy for prostate cancer recurrence after surgery. Noted study challenges include the limited availability of samples from NRG/RTOG 9601 and ability of available samples to meet quality control requirements (22.4% of total trial samples did not pass quality control), as the median age of tissue samples was older than 20 years.

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

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

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

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

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

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

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

metastases was 0.76, increasing to 0.81 with addition of Decipher results. The researchers concluded that Decipher GC has the ability to improve prognostication for individuals with prostate cancer post-prostatectomy and recommend ongoing study of the best methods of incorporating this type of testing into clinical practice. ~~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.~~

~~ClassGlass 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.

~~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.~~

~~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.~~ 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 that despite~~in spite 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.

~~Shore et al. (2014) evaluated the clinical utility of the CCP score in a U.S.-based clinical setting. Urologists who participated in a prospective clinical study were sent a retrospective questionnaire to assess the value of the CCP test results. Fifteen urologists participated in the study, representing 15 distinct urology group practices. Questionnaires were received for 294 evaluable patients. All patients had localized prostate cancer. Physicians found the CCP score valuable and indicated that 55% of tests generated a mortality risk that was either higher or lower than expected. Physicians also indicated that 32% of test results would lead to a definite or possible change in treatment. The data suggest that the test would have the net effect of shifting patients from more aggressive treatment to more conservative treatment. This was evidenced by the significant association between change in treatment and lower CCP scores. Results of this survey study provide only indirect evidence of clinical utility as the study measured the likelihood of change in treatment as estimated by the physician, not the actual change in~~

~~treatment. The authors concluded that real-world use of the test is likely to lead to a change in treatment in a significant portion of tested patients, particularly by shifting patients towards more conservative management.~~

~~Crawford et al. (2014) conducted a prospective survey study evaluating the impact of the CCP score on physician treatment recommendations for prostate cancer. Physicians ordering the test completed surveys regarding treatment recommendations before and after they received and discussed test results with patients. Clinicians also rated the influence of the test result on treatment decisions. For patients originally targeted for interventional therapy, results of the CCP test led to a 37.2% reduction of interventional therapy. For patients originally targeted for noninterventional therapy, 23.4% of patients had treatment changes to interventional therapy based on test results. Overall, surgical interventions were reduced by 49.5%, and radiation treatment was reduced by 29.6% Author-reported limitations included physician selection of patients for testing, no evaluation of patient input in therapeutic choice and other potential treatment decision factors not queried by the survey. Results of this survey study provide only indirect evidence of clinical utility since it does not capture clinical 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.

~~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.~~

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~~profiling of biopsies ~~may be considered in men with low or~~and ~~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.) . However, NCCN cautions that these tests (Decipher, Oncotype Dx Prostate, Prolaris or ProMark) have been developed with extensive industry support, guidance and involvement and have been marketed under the less rigorous FDA regulatory pathway for biomarkers. In addition, full assessment of their clinical utility requires prospective, randomized clinical trials. (NCCN, 2019g).~~

~~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

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

Tosoian et al. (2021) sought to validate an optimal threshold for the use of the MyProstateScore test in ruling out grade group ≥2 cancer in individuals referred for prostate biopsy. In this study, men who had not yet received prostate biopsy provided

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

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

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

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

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

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

among men with elevated PSA levels and could reduce the total number of unnecessary biopsies.

~~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~~Professional Societies~~

~~American Urological~~ 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~~ (AUA) with the American~~ Society of Clinical~~for Radiation~~ Oncology (ASCO~~ASTRO) and the Society for Urologic Oncology (SUO~~)

Eggener~~Sanda~~ et al. (2020~~2018~~) 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~~joint AUA/ASTRO/SUO guidelines~~ for select men to improve management decisions. There is a paucity of prospective studies assessing short- and long-term outcomes of patients when these markers are integrated into clinical decision making."

ASCO made four specific recommendations:

- Commercially available molecular biomarker tests (i.e., Oncotype Dx Prostate, Prolaris, Decipher, and ProMark) may be offered in situations in which the assay result, when considered as a whole with routine clinical factors, is likely to affect management. Routine ordering of molecular biomarkers is not recommended (Type: Evidence~~localized prostate cancer. The guidelines stated that tissue~~ based; Evidence quality: Intermediate; Strength of recommendation: Moderate).
- Any additional molecular biomarkers evaluated do not have sufficient data to be clinically actionable or are not commercially available and thus should not be offered (Type: Evidence based; Evidence quality: Insufficient; Strength of recommendation: Moderate).
- **Consideration of a commercially available molecular biomarker test (e.g., Decipher Genomic Classifier) is recommended in situations in which the assay result, when considered as a whole with routine clinical factors, is likely to affect management. In the absence of prospective clinical trial data, routine use of genomic biomarkers in the postprostatectomy setting to determine adjuvant versus salvage radiation or to initiate systemic therapies should not be offered (Type: Evidence based; Evidence quality: Intermediate; Strength of recommendation: Moderate).**
- In men with newly diagnosed prostate cancer eligible for ~~have not shown a clear role in~~ 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 localized prostate cancer and are not necessary~~ 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).~~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 Urological Association (AUA)/American Society for Radiation Oncology (ASTRO)

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

- **Clinicians may use tissue-based genomic biomarkers selectively when added risk stratification has the potential to impact clinical decision-making. (Expert Opinion)**
- **Clinicians should not use tissue-based genomic biomarkers routinely for risk stratification or to assist with clinical decision-making. (Moderate Recommendation; Evidence Level: Grade B)**

-
- **Patient and tumor risk factors should be fully assessed to guide decision regarding offering germline testing which would include mutations that are known to be associated with aggressive prostate cancer types or are known to have implications for treatment. (Expert Opinion)**

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

American Urological Association (AUA)/ ~~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 clinically localized prostate cancer. The guidelines stated that tissue based genomic biomarkers have not shown a clear role in active surveillance for localized prostate cancer and are not necessary for follow up.

~~American Urological Association (AUA)~~

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~~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:~~

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In 2018, Bekelman et al. ~~(2018)~~ published the ASCO endorsement of **the AUA/ASTRO/SUO** ~~their~~ 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).

~~*Lung Cancer*~~

National Comprehensive Cancer Network (NCCN)

NCCN clinical practice guidelines for prostate cancer (NCCN~~,~~ Prostate C~~e~~ancer, **v1.**202~~2~~3) 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~~**should be considered if not previously performed** to inform adjuvant therapy when adverse features are found post prostatectomy and can be part of the discussion of risk stratification in patients with prostate specific antigen ~~resistance~~persistence/recurrence after radical prostatectomy (category 2B evidence.)

~~In the~~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."

NCCN categorizes prostate cancer risk groups as follows:

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

Pancreatic Cancer and Ampullary Adenocarcinoma

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

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

Although current guidelines recommend somatic genomic sequencing for advanced pancreatic cancer patients, the benefit of this testing remains unclear. A 2021 systematic review and meta-analysis (Meti et al.) found that genomic sequencing can frequently identify targetable alterations in pancreatic cancer. In this review, 19 prospective studies of pancreatic cancer patients were analyzed. Each study conducted genomic sequencing to assist with clinical treatment selection. Methodologies for sequencing, definitions of targetable alterations and treatment selection approaches varied across studies and were unfortunately not completely reported. Of 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.

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

to be able to directly compare or combine the diagnostic performance of the methylation assay relative to genomic mutations. Further investigation with prospective evaluation is needed to determine the clinical relevance of these findings.

~~NCCN guidelines for NSCLC Panel have added a section on Plasma Cell-Free/Circulating Tumor DNA Testing that 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, 2019c).~~

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

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

65% sensitivity and 100% specificity. By NGS, the combination of *KRAS/GNAS* mutations and alterations in *TP53/PIK3CA/PTEN* had an 89% sensitivity and 100% specificity for advanced cancer. The study concluded that in comparison to Sanger sequencing, preoperative NGS of PCF for *KRAS/GNAS* mutations is highly sensitive for IPMNs and specific for mucinous PCs. In addition, the combination of *TP53/PIK3CA/PTEN* alterations is a useful preoperative marker for advanced cancer.

Lowery et al. (2018) performed comprehensive germline testing (GT) in a cohort of patients with exocrine pancreatic neoplasms. The genotype and phenotype associations were used to identify biomarkers for therapy response. Six hundred fifteen patients were prospectively tested for somatic tumor and matched sample profiling for 410-468 genes. PGAs were present in 122 (19.8%) of 615 patients involving 24 different genes, including *BRCA*1/2, *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 *ELF*3. Forty-five patients with pathologically confirmed AC were tested with the Memorial Sloan Kettering Integrated Mutation Profiling of Actionable Cancer Targets (MSK-IMPACT) test (410-468 genes). Twenty-three patients were also tested with GT with MSK-IMPACT (76-88 genes). Eight of 44 patients (18%) were identified as harboring pathogenic mutations in *BRCA2*, *ATM*, *RAD50*, and *MUTYH*. Additionally, they found a wide spectrum of SAs in genes such as *KRAS*, *MDM2*, *ERBB2*, *ELF3*, and *PIK3CA*. Two patients in the cohort underwent SA-targeted therapy, and 1 had a partial radiographic response.

Clinical Practice Guidelines

~~Professional Societies~~

American Society of Clinical Oncology (ASCO ~~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;~~
  - ~~Nonsquamous, non-small-cell histology;~~
  - ~~Any non-small-cell histology when clinical features indicate a higher probability of an oncogenic driver (e.g., young age [< 50 years]; light or absent tobacco exposure).~~

- ~~BRAF testing should be performed on all patients with advanced lung adenocarcinoma, irrespective of clinical characteristics. RET, or KRAS, or MET molecular testing are not recommended as single gene routine stand-alone assays outside the context of a clinical trial. It is appropriate to include these as part of larger testing panels performed either initially or when routine EGFR, ALK, BRAF, and ROS1 testing is negative.~~
- ~~Multiplexed genetic sequencing panels are preferred where available over multiple single gene tests to identify other treatment options beyond EGFR, ALK, BRAF, and ROS1.~~
- ~~Circulating tumor cell free DNA testing, also called a liquid biopsy, should not be routinely considered due to lack of evidence of efficacy. However, the expert consensus opinion provided is that cfDNA may be used in some clinical settings in which tissue is limited and/or insufficient for molecular testing to identify EGFR mutations.~~

~~National Comprehensive Cancer Network (NCCN)~~

~~NCCN guidelines for NSCLC (NCCN, 2017) strongly endorse the use of broad molecular profiling (also known as precision medicine) to detect certain rare mutations using multiplex or NGS. Presence of EGFR-activating mutations represents a critical biological determinant for proper therapy selection in patients with lung cancer, stating "determination of the specific molecular abnormalities of the tumor is critical for predicting sensitivity or resistance to an increasing number of druggable targets, primarily tyrosine kinase inhibitors (TKIs)". Data has shown that targeted therapy is potentially very effective in patients with specific gene mutations or rearrangements. The guidelines specifically report that "EGFR and ALK testing be conducted as part of broad molecular profiling." The NCCN Panel states that such testing would ensure that patients receive the most effective available targeted treatment for NSCLC.~~

~~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.~~

~~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 Dimston, 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).~~

~~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 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.~~

~~Partyka et al. (2018) recently conducted a small retrospective study on 10 archived FNA samples comparing two commercially available miRNA tests, ThyraMir and RosettaGxReveal. The samples represented follicular lesion of undetermined significance (FLUS, n = 5), follicular neoplasm/suspicious for follicular neoplasm (FN/SFN, n = 4), and suspicious for malignancy (SM, n = 1). Of the seven cases with benign histology, six smears were classified as benign by the RosettaGX microRNA classifier, and one case was designated as suspicious. RosettaGX showed a 75% positive predictive value in comparison to 60% for ThyGenX/ThyraMIR, and both tests demonstrated a 100% NPV.~~

~~Next-generation-sequencing (NGS) tests that identify variants in genes associated with thyroid cancer have also been used to help resolve the clinical dilemma presented by indeterminate cytology on thyroid nodules. At least seven genes have found to have a high degree of specificity for thyroid malignancy, including BRAF, RAS, HRAS, and NRAS mutations and the gene fusions RET/PTC1, RET/PTC3 and PAX8/PPARγ (Zhang and Linm 2016, Beaudenon-Huibregtse et al., 2014). Rapid technological advances have allowed laboratories the opportunity to add many more genes to their sequencing platforms and may additionally analyze micro-RNA simultaneously. For example, the ThyroSeq v3 assay analyzes 112 genes, providing information on > 12,000 mutation hotspots and > 120 gene fusion types. In a publication describing the validation of the assay, Nikiforova et al. (2018) reported that in a training set of 238 tissue samples and 175 FNA samples with known surgical follow-up, the test was able distinguish cancer from benign tissue nodules with 93.9% sensitivity, 89.4% specificity, and 92.1% accuracy. In FNA the authors report a sensitivity of 98.0%, a specificity was 81.8%, and accuracy of 90.9%. Additional studies are necessary to determine the real world analytical validity and clinical utility of this test.~~

~~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 alterations. 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 16 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) guidelines for Thyroid Carcinoma (NCCN, 2019i) recommend molecular profiling for thyroid nodules with indeterminate or suspicious for follicular neoplasm cytology. They note to use molecular markers with caveat and caution. Molecular profiling is not recommended for Hurthle cell neoplasms. Molecular testing of single genes, especially BRAF, or a multigene panel that includes BRAF, NRAS, HRAS, KRAS,RET/PTC1, RET/PTC3, and PAX8/PPARγ or a gene expression classifier test may be considered, and should be selected by the clinician based on the clinical question being asked.~~

~~Professional Societies~~

~~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 suggest 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, and 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, seven 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 three with myelodysplastic syndrome (MDS). Cytogenetic analysis revealed CNV in 11 regions in eight patients. SNP + CGH found 14 CNV in nine patients, and cryptic LOHs in three of five patients with normal cytogenetics. Overall, nine patients were found to have abnormalities not detected by routine cytogenetics. Three 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. Ten regions of copy number variation was 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.~~

~~Professional Societies~~

~~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 gene expression profiling (GEP) has the potential to provide additional prognostic value to further refine risk-stratification, help therapeutic decisions and inform novel drug design and development. The NCCN panel unanimously agreed that although GEP is not routinely used in clinical practice during diagnostic workup, it may be helpful in selected patients to estimate the aggressiveness of the disease and individualize treatment. No patient selection criteria were provided (NCCN, 2019d).~~

~~Detection of Minimal Residual Disease (MRD) in Hematologic Malignancies~~

~~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.~~

~~Avet-Loiseau et al. (2015) reported on the use of FC and NGS in the Intergroupe 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.~~

~~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 51.4%, and were highly variable across the genes analyzed. DTA mutations were most common, persisting at rates of 78.7%, 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 oligomuccelotide 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 TRC 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 (38.7%), 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.~~**Sohal et al. published an update to the ASCO Metastatic Pancreatic Cancer Guideline in 2020, noting that a complete discussion of molecular biomarker testing is outside the scope of the guideline, but a modification to the recommendations around molecular testing was made. This includes recommendation that all patients with pancreatic cancer should be offered information about biomarker testing and biomarker testing (specifically *NTRK* fusion testing) should be used in patient selection for targeted therapies.**

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

National Comprehensive Cancer Network (NCCN)

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

~~Determining the response to treatment is an important aspect of managing multiple myeloma, and NCCN guidelines recently adding assessing MRD to the management algorithm. 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 for individuals that have undergone autologous or allogenic bone marrow transplant. 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, 2018a). NCCN does not~~

have the same stance on using NGS for MRD detection in AML (NCCN AML 2019). They note that the use of MRD as a management or prognostic tool for AML is still emerging, and that common assessments are RT-PCR for NPM1, CBFB-MYH11, and RUNX1-RUNX1t1 and flow cytometry. The sensitivity of these two approaches is considered superior at this time to NGS using targeted panels of 20-50 genes.

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, for 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 (KeramatiniaBreast cancer (Cancsan et al., 20172014; Wheler et al., 2014)
- 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)
- Head and neck cancer (Wang et al., 2017; Chung et al., 2015)
- 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 many of 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 = 191, 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)~~

~~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.~~

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

Solid Tumor Tissue Testing

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

cancer types included colorectal cancer, lung cancer, melanoma, gastric cancer and pan-cancer. Based on the results of this analysis, the researchers concluded that TMB shows promise for use as a biomarker for immunotherapy treatment. They recommend establishing a standard for TMB assessment including cut-off values, to improve management of various cancer types.

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

A 2022 Hayes Precision Medicine Insight report found some support (based on review of 12 abstracts only) for comprehensive molecular profiling (CMP) of solid tumors when used to broadly profile tumor tissue and provide assistance with selection of matched therapy specific to the identified biomarkers. Hayes notes that support from professional guidelines for use of CMP in this manner is weak, citing one guideline indicating NGS may be used in some situations and two guidelines that address the need for appropriate infrastructure interpretation and implementation of test results as well as quality assurance. The report specifically notes that the use of CMP to test for specific biomarkers with associated FDA-approved, cancer-specific therapies was not addressed in this report (Hayes, Comprehensive Molecular Profiling Test(s) for Solid Tumors Intended to be Used as Broad Molecular Profiling Tool to Assigned Matched Therapy, 2022).

In a comparative study, Ramos-Paradas et al. (2021) assessed two marketed NGS panels used for TMB evaluation in NSCLC. TruSight Oncology 500 (TSO500) and Oncomine Tumor Mutation Load (OTML) were compared to a reference assay (FoundationOne [FO]) in samples from 96 participants with NSCLC. Agreement in PD-L1 expression and level of various immune infiltrates compared to TMB were also assessed and an inter-laboratory reproducibility study was performed. Ultimately, determination was made regarding adjusted cut-off values to be used. Concordance correlation coefficients (CCC) were 0.933 (95% CI 0.908 to 0.959)

for TSO500 and 0.881 (95% CI 0.840 to 0.922) for OTML, indicating strong agreement with FO. Corresponding CCCs in tumors with <1% of cells expressing PD-L1 (PD-L1<1%; N=55) were 0.951 (TSO500-FO) and 0.919 (OTML-FO). In tumors with PD-L1 ≥1% (N=41), corresponding CCCs were 0.861 (TSO500-FO) and 0.722 (OTML-FO). TSO500 had higher reproducibility in the inter-laboratory reproducibility analyses and no significant differences were noted in immune infiltration compared to TMB. To guarantee sensitivity >88%, adjusted cut-off values corresponding to 10 mut/Mb with FO needed to be lowered to 8.380 mut/Mb for OTML) and 7.847 mut/Mb for TSO500). Using these cutoff values, the positive predictive value (PPV) for TSO500 was 78.57% (95% CI 67.82 to 89.32) and the negative predictive value was 87.50% (95% CI 77.25 to 97.75) for TSO500 and the PPV for OTML was 73.33% (95% CI 62.14 to 84.52) and negative predictive 86.11% (95% CI 74.81 to 97.41). These study findings led to the conclusion that both TSO500 and OTML showed strong analytical performance for assessment of TMB. Concordance was stronger in those individuals with negative PD-L1 expression, and TSO500 demonstrated higher inter-laboratory reproducibility.

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

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

The TRITON2 trial was an international open-label phase II study assessing the use of rucaparib in individuals diagnosed with metastatic castration-resistant prostate cancer (mCRPC) associated with a mutation in BRCA or another homologous recombination-directed DNA damage repair (DDR) gene who had progressed subsequent to treatment with next-generation androgen receptor (AR)-directed therapy and taxane-based chemotherapy. Abida et al. (2020) reported on results of this study related to mCRPC associated with a BRCA mutation that was treated with rucaparib twice daily as part of the TRITON2 study. Key outcomes included ORR per RECIST as determined by blinded, independent radiology reviewers and investigators and locally assessed PSA response rate. The population under review was comprised of 115 individuals with a BRCA gene alteration that did or did not have measurable disease. Confirmed ORRs were 43.5% (95% CI, 31.0% to 56.7%; 27 of 62 participants) for those with measurable disease and 50.8% (95% CI, 38.1% to 63.4%; 33 of 65 participants) for those without measurable disease. PSA response rate was 54% (95% CI, 45.2% to 64.1%; 63 of 115 participants). Consistent ORRs were seen in individuals with germline or somatic BRCA alterations and for those individuals with a BRCA1 or BRCA2 alteration. A higher PSA response rate was seen, however, in those individuals with BRCA2 alterations. The authors concluded that data from the TRITON2 study highlight the importance of use of genomics in the identification of individuals that may benefit from treatment with a PARP inhibitor and are consistent with results of other studies on PARP inhibitors and their association with mCRPC and BRCA alterations. Although no control arm was present in this study and OS data is limited so far, the researchers assert that the TRITON2 study results support the importance of the antitumor impact of rucaparib in individuals with mCRPC and a detrimental BRCA mutation while maintaining a manageable safety profile.

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

FoundationOne CDx

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

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

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

Trédan et al~~.~~. (2019) studied the impact of molecular profiling on adult and pediatric patients with solid or hematological advanced cancer that was previously treated in advanced/metastatic settings. The profile was performed on tumors, relapse or biopsies and then reviewed by a molecular tumor board to determine if any molecular-based therapies were available. At four different institutions, 2,579 patients were enrolled, and the tumor board reviewed 1,980 patient molecular profiles. There were some genes determined to be most frequently altered and those included: CDKN2A (n = 181, 7%), KRAS (n = 177, 7%), PIK3CA (n = 185, 7%), and CCND1 (n = 104, 4%). A molecular-based therapy was recommended for 699/2579 patients (27%), however only 163/2579 patients (6%) received at least one MBRT. Likewise, out of the 182 lines of therapy initiated, 23 (13%) partial responses were observed. Overall, only 0.9% of the whole cohort experienced an objective response. The researchers concluded that molecular screening should not be used at present to guide clinical decision-making outside of a clinical trial.

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

in the clinical course and expanded access to genomically guided early-phase clinical trials and targeted agents may increase clinical application.

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

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across alteration types, with high specificity (positive predictive value [PPV] > 99%). The authors confirmed accuracy using 249 FFPE cancer specimens characterized by established assays. Application of the test to 2,221 clinical cases revealed clinically actionable alterations in 76% of tumors, three times the number of actionable alterations detected by current diagnostic tests. This study did not evaluate the clinical utility of such findings in improving care and outcome of patients by tailoring treatments or predicting response to treatment. Hence, it is important to note that the clinical utility of genomic profiling using massively parallel DNA sequencing remains unknown. In addition, study authors colleagues did not categorize the data regarding sensitivity, specificity, and positive predictive value (PPV) by cancer type.

FoundationOne Heme

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

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

MI Profile and MI Tumor Seek (Caris Life Sciences)

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

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

~~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.~~

~~Another COMPASS trial publication described the use of real-time WGS and RNASeq of advanced PDAC to identify predictive mutational and transcriptional features for better treatment selection (Aung et al., 2018). Sixty-three patients underwent a tumor biopsy and WGS and RNASeq were successful in 62 (98%) and 60 (95%), respectively. PDAC RNA subtypes were compared to basal-like subtypes for chemotherapy response. GATA6 expression in tumor measured by RNA in situ hybridization was found to be a robust surrogate biomarker for differentiating classical and basal-like PDAC subtypes. These potentially actionable genetic alterations were found in 30% of patients.~~

~~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.~~

~~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.~~

~~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 *BRCA*1/2, *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.~~

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.

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

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

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

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

a localized recurrence. The final two of the eight individuals with positive MRD had not had clinical recurrence at their last follow-up visit. For individuals with high-risk HR+ breast cancer greater than 5 years from initial diagnosis, the researchers found that ctDNA was identified approximately one year before all cases of distant metastasis in this study. Further high-quality studies are needed to determine if ctDNA-guided interventions will ultimately impact clinical outcomes for individuals with cancer.

A ~~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 (Hayes, Liquid Biopsy Tests for Colorectal Cancer Screening, 2020, updated 2022).**~~.~~

Petit et al~~.~~**. (2019) performed a systematic review to determine the evidence available regarding ctDNA as a screening tool for colorectal cancer. After review, 69 studies were included and 17 studies reviewed total cell free DNA, six studies looked at the DNA integrity index and 15 focused on ctDNA. While the researchers concluded that ctDNA is a promising candidate for colorectal cancer screening, further researcher is required.** ~~(2017) and Su et al. (~~

~~Another~~ study on 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.~~

A~~nother~~ **study by Lam et al. (2019, included in Hayes Guardant360 [Guardant Health Inc.], 2018) studied lung squamous-cell carcinoma (LUSC) and cfDNA. The researchers retrospectively evaluated 492 LUSC patients: 410 patients (stage 3B or 4 LUSC) were tested with a targeted cell-free circulating DNA NGS assay and 82 patients (any stage) were tested with a tissue NGS cancer panel. Overall, 467 patients (95%) had a diagnosis of LUSC, and 25 patients (5%) had mixed histology. Of the LUSC subgroup, a total of 11% had somatic alterations with therapeutic relevance in the cfDNA testing, including in *EGFR* (3%), *ALK/ROS*1 (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 In~~V~~VisionFirst** 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 identify patients. Eighty-eight patients with 96 plasma-detected ALK fusions were determined. The fusion partners identified included *EML4* (85.4%), *STRN* (6%), and *KCNQ5, 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 ctDNA using the Guardant360 test. In this cohort, mutations in TP53, EGFR, and KRAS genes were most often determined. Mutations in BRCA1, BRCA2, and ATM were found in 18.1% (32/177) of cases. Also, the researchers compared the ctDNA and tumor tissue of 37 lung cancer cases. This analysis found that key mutations could be found in plasma even if they were minor in the tumor tissue.~~

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

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

genotyping, patients with T790M-negative plasma results still need a tumor biopsy to determine presence or absence of T790M.

~~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 nonsquamous 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-biopsyrebiopsy. 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.~~

FoundationOne Liquid CDx

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

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

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

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

as a method to assist with clinical decision-making in individuals with *ALK*-positive NSCLC.

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

~~Galleri~~

Galleri

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

Klein et al. (2021) documented the results of an observational study to validate a multi-cancer early detection test designed to complement existing screening methods and potentially increase the number of cancers found through population screening, potentially impacting and improving clinical outcomes. Including 4077 participants in an independent validation set (cancer n = 2823, non-cancer n = 1254), sensitivity, specificity and cancer signal origin (CSO) were measured. This population was a pre-specified sub-study of the Circulating Cell-free Genome Atlas Study, a prospective, multi-center, observational study designed to collect biological samples (blood and tumor tissue) both from participants with newly diagnosed cancer and from participants without a diagnosis of cancer to characterize population heterogenicity in cancer and cancer-free participants so that models for distinguishing between cancer and non-cancer could be developed. According to the authors, the Atlas study demonstrated that MCED testing using cfDNA in combination with machine learning could detect cancer signals across various cancer types and predict cancer signal origin with high accuracy. The objective of the current study is to further validate an MCED test that has been refined for use as a screening tool. Overall sensitivity for cancer signal detection was 51.5% and showed increasing sensitivity with stage of cancer. Cancer signal detection specificity was 99.5% (95% confidence interval). Cancer signals were detected across more than 50 cancer types. CSO prediction in true positives was 88.7% overall. The researchers concluded that the MCED test showed high specificity and accuracy in prediction of CSO and detected signals across multiple cancer types. A noted limitation is that blood sample collection from participants with cancer done after biopsies had been performed could increase the possibility that tumor cfDNA fraction could also increase relative to pre-biopsy. In addition, CCGA is a case-control study, so would not reflect performance in a screening population. Further studies evaluating test performance and clinical utility in target-use population are needed.

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

Guardant 360 **CDx**

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

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

In 2022, Bauml (included in the Palmieri systematic review above) assessed the clinical validation of Guardant 360 CDx as a blood-based companion diagnostic for sotorasib to detect *KRAS* p. G12C (an oncogenetic non-small cell lung cancer driver mutation). The primary aim of the current analysis was to evaluate the clinical validity of Guardant360 CDx via data and samples from the CodeBreaK100 (NCT03600883) study. The secondary purposes were to evaluate the concordance among *KRAS* p.G12C mutation status decided by

the therascreen® *KRAS* RGQ PCR kit and Guardant360 CDx in individuals with NSCLC; to assess the representativeness of the Guardant360 CDx-positive cohort related to the entire analysis group. And to consider DOR, DCR, and time to response (TTR) in individuals with *KRAS*p.G12C-mutant NSCLC as detected by Guardant360 CDx comparative to the whole analysis group. The ORR (95% CI; individuals with objective response/all individuals in the dataset) for all individuals was 37.1% (28.6%, 46.2%; n = 46/124) in the Full Analysis Set, 36.4% (25.7%, 48.1%; n = 28/77) in the Guardant360 positive cohort, and 46.7% (28.3%, 65.7%; n = 14/30) in the Guardant360 negative cohort. Rates of PD, SD, and PR were similar among the cohorts, with SD being the most common outcome (Full Analysis Set, n = 54/124 [43.5%]; Guardant360 Evaluable, n = 46/107 [43.0%]; Guardant360 positive, n = 32/77 [41.6%]; Guardant360 negative, n = 14/30 [46.7%]). DCR (95% CI; individuals with disease control/all those in the dataset) was 80.6% (72.6%, 87.2%; n = 100/124) in the Full Analysis Set and 77.9% (67.0%, 86.6%; n = 60/77) in the Guardant360 positive cohort. Among responders, DOR was ≥ 3 months in 38/46 (82.6%) those in the Full Analysis Set and 24/28 (85.7%) individuals in the Guardant360 positive cohort; DOR was ≥ 6 months in 28/46 (60.9%) and 15/28 (53.6%) those in the Full Analysis Set and Guardant360 positive cohort, respectively. Of the four cohorts, DOR ≥ three months among responders was numerically highest in the Guardant360 positive cohort (n = 24/28 [85.7%]), while DOR ≥ 6 months was mathematically highest in the Guardant360 negative (n = 9/14 [64.3%]) cohort. The average time to objective response was comparable between all cohorts. The authors concluded that liquid biopsy using Guardant360 CDx has clinical validity for the identification of individuals with *KRAS*p.G12C-mutant NSCLC and, amplified by tissue testing methodologies, will identify individuals for treatment with sotorasib.

~~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 CT (DNAs) 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.~~

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~~6~~ (33%) had ROS1 kinase domain mutations (five~~5~~ 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.

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

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

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

~~Another study by Lam et al (2019) studied lung squamous-cell carcinoma (LUSC) and cfDNA. The researchers retrospectively evaluated 492 LUSC patients with 410 patients (stage 3B or 4 LUSC) who were tested with a targeted cell-free circulating DNA NGS assay, and 82~~

~~patients (any stage) who were tested with a tissue NGS cancer panel. Overall 467 patients (94.9%) had a diagnosis of LUSC, and 25 patients (5.1%) had mixed histology. Of the LUSC subgroup, a total of 10.5% had somatic alterations with therapeutic relevance, including in EGFR (2.8%), ALK/ROS1 (1.3%), BRAF (1.5%), and MET amplification or exon 14 skipping (5.1%). 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.~~

~~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.~~

~~One liquid biopsy test, Guardant360, evaluates cell-free tumor DNA for 73 different genes. The~~ The majority of studies with Guardant360 have focused on NSCLC; however, more research is being performed with other tumor types. A study by Yang, et al. (2017) evaluated lung cancer and other solid tumors. Plasma from patients with lung cancer (n = 103) and other solid tumors (n = 74) was analyzed for ctDNA using the Guardant360 test. In this cohort, mutations in TP53, EGFR, and KRAS genes were most often determined. Mutations in BRCA1, BRCA2, and ATM were found in 18.1% (32/177) of cases. Also, the researchers compared the ctDNA and tumor tissue of 37 lung cancer cases. This analysis found that key mutations could be found in plasma even if they were minor in the tumor tissue.

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

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

The use of ~~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 individuals~~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~~for individuals with early breast cancer. A retrospective ancillary ctDNA study was performed on samples that had been prospectively collected from high-risk individuals with early breast cancer ~~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~~ Participants with *de novo* metastatic disease were not included in the study. In addition, eligibility was limited to individuals who had received a MammaPrint high score. On pretreatment testing, 73% of participants were ctDNA positive. Those participants who continued to be ctDNA positive 3 weeks after initiation of paclitaxel were significantly more likely to have residual disease after neoadjuvant chemotherapy (NAC) when compared to those who were no longer ctDNA positive. All individuals ~~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~~ individuals were cleared of disease. In the post-therapy period, ctDNA-positive ~~patients~~ participants were more than 40 times more likely to have a recurrence of their disease than the ctDNA-negative ~~patients~~participants. Actionable mutations were found in 81.8% of the relapse samples that were ctDNA-positive. The researchers concluded that ctDNA analysis has potential to be helpful with postoperative management of CRC, in terms of early relapse detection, ACT monitoring and risk stratification. However, the sample size of participants with recurrent CRC in this study was small and analysis was done on multiple different ~~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)

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

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

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

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

~~InVisionFirst Lung~~

~~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 can't 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.~~

~~The NCCN NSCLC Guidelines Panel for NSCLC have added a section on Plasma Cell-Free/Circulating Tumor DNA Testing that 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.~~

~~In a 2017 study, Cohen et al. developed 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.~~

~~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 ct-DNA in RCC patients.~~

~~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 researched concluded that this study shows the clinical utility of ctDNA for prognosis and disease monitoring in RCC.~~

~~Professional Societies~~

~~American Society of Clinical Oncology (ASCO)~~

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

clinical utility and little evidence of clinical validity of ctDNA tests in early-~~-~~stage cancer, treatment monitoring, or residual disease detection. Likewise, no evidence of clinical validity and utility was demonstrated in the literature review for the use of ctDNA in cancer screening.

The European Society for Medical Oncology (ESMO)

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

International Association for the Study of Lung Cancer (IASLC)

In a 2021 Consensus Statement from the ~~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.

National Institute for Health and Care Excellence (NICE)

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

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

specific cancers and metastatic cancer of unknown primary origin, and no evidence of its use in children.

National Comprehensive Cancer Network (NCCN)

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

Ampullary Adenocarcinoma

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

Bladder Cancer

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

Bone Cancer

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

~~Breast Cancer~~

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

Cervical Cancer

For persistent or recurrent cervical cancer, NCCN indicates

- CGP via a validated and/or FDA approved assay should be considered

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

Colorectal Cancer (CRC)

The NCCN guidelines for Colon Cancer and Rectal Cancer indicate that targeted treatment for advanced/metastatic CRC is becoming more common and as such, NCCN has expanded recommendations for biomarker testing. For individuals with metastatic CRC, recommended workup should include determination of tumor gene status for *RAS* and *BRAF* mutations, HER2 amplifications, and MSI/MMR status (if not previously done) either individually or as part of tissue- or blood-based NGS panel test. NGS panels have the advantage of the ability to detect rare and actionable gene alterations such as *NTRK* fusions. The guideline further notes that molecular testing on tissue samples is preferred, but blood-based assays are also an option. Both tissue- and blood-based NGS panels have the ability to pick up rare and actionable mutations and fusions. (NCCN Colon Cancer, v2.2022, NCCN Rectal Cancer, v3.2022)

Histiocytic Neoplasms

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

Gastric and Esophageal/Esophagogastric Junction Cancers

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

Melanoma: Cutaneous

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

Non-Small Cell Lung Cancer

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

- patient is not medically fit for invasive tissue sampling
- In the setting of initial diagnosis, if following pathologic confirmation of a NSCLC diagnosis there is insufficient material for molecular analysis, cell-free/circulating tumor DNA should be used only if follow-up tissue-based analysis is planned for any patient in which an oncogenic driver is not identified

- In the setting if initial diagnosis, if tissue-based testing does not fully assess all recommended biomarkers due to quantity of tissue available or testing methodologies available, consider repeat biopsy and/or cell-free/circulating tumor DNA testing
- In advanced or metastatic disease, If there is not enough available tissue to allow testing for genes including *EGFR*, *KRAS*, *ALK*, *ROS1*, *BRAF*, *NTRK*1/2/3, *MET*, *RET*, and *ERBB2* (HER2) repeat biopsy and/or plasma testing should be done.

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

Occult Primary

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

Ovarian Cancer/Fallopian Tube Cancer/Primary Peritoneal Cancer

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

Pancreatic Adenocarcinoma

NCCN recommends tumor/somatic molecular profiling for individuals with locally advanced/metastatic disease who are candidates for anti-cancer therapy for identification of uncommon mutations. Testing for potentially actionable somatic findings including, but not limited to: fusions (*ALK*, *NRG1*, *NTRK*, *ROS1*, *FGFR2*, *RET*), mutations (*BRAF*, *BRCA*1/2, *KRAS*, *PALB2*), amplifications (*HER2*), microsatellite instability (MSI), and/or mismatch repair (MMR) deficiency should be considered. Of note, testing on tumor tissue is preferred; however, cell-free DNA testing can be considered if tumor tissue testing is not feasible. (NCCN Pancreatic Adenocarcinoma, v1.2022)

Prostate Cancer

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

U.S. Food and Drug Administration (FDA)

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

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

Date	Summary of Changes
TBD	**Application** • **Added language to indicate any requests for services that do not meet criteria set in the Prior Authorization Review Panel (PARP) will be evaluated on a case-by-case basis; refer to *Pennsylvania Exceptions, Pennsylvania Code, Title 55, Chapter 1101*** **Coverage Rationale** ***State-Specific Criteria*** • **Added language to indicate the coverage criteria for genetic counseling contained in this policy represents the Louisiana Medicaid Managed Care Organization Manual (LA MCO) coverage policy and is set forth below in accordance with State requirements** **Genetic Counseling** • **Added language to indicate genetic counseling before and after all genetic testing is required; counseling must consist of at least all of the following and be documented in the medical record:** ○ **Obtaining a structured family genetic history;** ○ **Genetic risk assessment; and**

	○ Counseling of the enrollee and family about diagnosis, prognosis, and treatment **Breast Cancer** • Revised language to indicate: ***Oncotype DX (Breast Cancer Assay)*** ○ Coverage criteria for Oncotype DX Breast Cancer Assay for the determination of breast cancer prognosis includes: ▪ Oncotype DX Breast Cancer Assay should be done within six months of the initial diagnosis of breast cancer • Oncotype DX Breast Cancer Assay should be considered for individuals only after surgery and subsequent pathological examination of the tumor has been completed ▪ Histology indicates the cancer is ductal, lobular, mixed, or metaplastic • Histology shows the cancer is not tubular or colloid ▪ Estrogen receptor is positive (ER+), or progesterone receptor is positive (PR+) or both • HER2 receptor is negative ▪ Chemotherapy is a therapeutic option being considered for treatment and will be supervised by the practitioner ordering the gene expression profile • Node negative or Node positive (1-3 nodes only) on individuals who are post-menopausal ***Gene Expression Profiling*** ○ Gene expression profiling as a technique of managing the treatment of breast cancer is considered investigational and not medically necessary when a gene profiling test other than Oncotype DX Breast Cancer Assay is being used, including but not limited to: ▪ Breast Cancer Gene Expression Ratio (also known as Theros H/I℠) • Breast Cancer Index℠ ▪ Insight® DX Breast Cancer Profile • MammaPrint® ▪ Mammostrat • Oncotype DX DCIS ▪ Pam50 Breast Cancer Intrinsic Classifier™ • The 41-gene signature assay ▪ The 76-gene "Rotterdam signature" assay • THEROS Breast Cancer Index℠ ○ Gene expression profiling as a technique of managing the treatment of ductal carcinoma in situ (DCIS) is considered investigational and not medically necessary under all circumstances ○ Repeat gene expression profiling with the Oncotype DX Breast Cancer Assay for the same tumor, such as a metastatic focus, or from more than one site when the primary tumor is multifocal is considered investigational and not medically necessary

Additional Non State-Specific Criteria

- Added coverage guidelines for the following indications (refer to the policy for complete details):
  - Prostate Cancer Gene Expression Profiling (GEP)
  - Companion Diagnostics via Tissue Sample for Solid Tumor Cancers
  - Companion Diagnostics via Plasma Sample/Liquid Biopsy (cell-free DNA [cfDNA] or circulating tumor DNA [ctDNA]) for Solid Tumor Cancers
- Replaced language indicating "molecular *profiling using* gene expression profiling, *Chromosome Microarray* multi-gene cancer panels is unproven and not medically necessary for all other indications [not listed as proven in the policy]" with "molecular *testing such as* gene expression profiling, multi-gene *NGS-panels, and CGP* is unproven and not medically necessary for all other indications [not listed as proven in the policy]"
- Revised list of unproven and not medically necessary molecular testing (profiling/panels) to reflect/include:
  - NGS panels of > 50 genes unless otherwise specified
  - Decipher® Bladder
  - ResponseDx Tissue of Origin™, CancerTYPE ID®, Rosetta Cancer Origin™, ProOnc
  - PancraGEN®, PancreaSeq®
  - Oncotype DX® colon cancer assay, Colorectal Cancer DSA™, Genefx℠ Colon (also known as ColDx), OncoDefender™-CRC, ColoPrint®
  - DecisionDx® Melanoma, DermTech PLATM, myPath®-Melanoma)
  - MyPRS®/MyPRS Plus™
  - Multi-cancer early detection/screening tests (e.g., Galleri®)
  - TMPRSS2 fusion gene, Prolaris® Prostate Cancer Test, ExoDX Prostate Test, MiPS (Mi Prostate Score Urine test), MyProstateScore (MPS, formerly MiPS), Confirm MDx, Select MDx
  - Tumor-informed assays (e.g., Invitae Personalized Cancer Monitoring, Signatera™, RaDaR®)
  - MRD monitoring for solid tumors (e.g., Guardant Reveal™)
  - Percepta® GSC for suspicious lung nodules
  - Solid tumor profiling that includes Whole Exome, Whole Genome or whole transcriptome Sequencing (e.g., Caris MI Tumor Seek™, Caris MI Profile™, Tempus xE)

Lung Cancer

- Replaced language indicating "*multigene* molecular profiling of non-small cell lung cancer is proven and medically necessary when all of the [listed] criteria are met" with "molecular profiling of *solid tumor tissue in metastatic* non-small cell lung cancer is proven and medically necessary when all of the [listed] criteria are met"
- Replaced reference to "Liquid Biopsy (circulating tumor DNA)" with "Liquid Biopsy [*cell-free DNA (cfDNA) or* circulating tumor DNA *(ctDNA)*]"

- Revised coverage criteria for:

  Molecular Profiling of Solid Tumor Tissue in Metastatic Non-Small Cell Lung Cancer

  - Removed criterion requiring "the 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"
  - Replaced criterion requiring "the panel selected has no more than 50 genes" with "the multigene *NGS* panel selected has no more than 50 genes *or the individual meets criteria for companion diagnostic testing [listed in the policy]*"

  Liquid Biopsy Molecular Profiling Tests of Non-Small Cell Lung Cancer

  - Removed criterion requiring:
    - Non-small cell lung cancer has been pathologically confirmed, but there is insufficient material available for molecular testing
      - 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
  - Replaced criterion requiring:
    - "The individual is not medically fit for invasive biopsy" with "the individual is not medically fit for invasive biopsy *or tumor tissue testing is not feasible*"
      - "The *test* selected has no more than 50 genes" with "the *multigene NGS panel* selected has no more than 50 genes *or the individual meets criteria for companion diagnostic testing [listed in the policy]*"
  - Removed language indicating 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) is unproven and not medically necessary due to insufficient evidence of efficacy

Thyroid Cancer or Indeterminate Thyroid Nodule Testing

- Revised language to indicate:
  - The use of GEP testing for thyroid nodules with indeterminate cytology [e.g., Afirma® Genomic Sequencing Classifier (GSC), ThyroSeq® V3, ThyGeNEXT®/ThyraMIR®] is proven and medically necessary when all of the following criteria are met:
    - Follicular pathology on fine needle aspiration is indeterminate (Bethesda III/IV)
      - The results of the test will be used for making decisions about further surgery
  - Due to insufficient evidence of efficacy, molecular tests for indeterminate thyroid nodules other than those previously described as proven are unproven and not medically necessary, including but not limited to:

	▪ Afirma® Xpression Atlas (XA) • Comprehensive Genomic Profiling (CGP) (e.g., NeoTYPE® Thyroid Profile) ○ The use of more than one molecular profile test in an individual with an indeterminate thyroid nodule is unproven and not medically necessary due to insufficient evidence of efficacy ○ CGP of confirmed anaplastic thyroid cancer is proven and medically necessary; for all other primary thyroid cancers, refer to the criteria for FoundationOne® CDx [in the policy] **Uveal Melanoma Gene Expression Testing (GEP)** • Added language to indicate 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 **Hematological Cancer Testing** • Revised language to indicate: ○ Clonality assessment at initial diagnosis (e.g., ClonoSeq® Clonality ID) on one specimen only is proven and medically necessary when ordered by a hematologist or oncologist for individuals with: ▪ Acute lymphoblastic leukemia • Multiple myeloma ○ The use of multigene panels (50 genes or fewer) at initial diagnosis is medically necessary when ordered by a hematologist or oncologist for individuals with: ▪ Acute lymphoblastic leukemia • Acute myeloid leukemia ▪ Multiple myeloma • Myelodysplastic syndrome suspected ▪ Myeloproliferative neoplasm ○ Measurable residual disease (MRD) testing (e.g., ClonoSeq® MRD) is proven and medically necessary when ordered by a hematologist or oncologist for individuals with all of the following: ▪ Acute lymphoblastic leukemia or multiple myeloma; and • Testing occurs after completing a course of therapy ○ Specific biomarker identification for hematologic cancers is considered medically necessary when biomarker confirmation is required per the *Indications and Usage* of the US FDA-approved prescribing label prior to initiation of therapy ○ CGP (e.g., FoundationOne Heme) for hematological malignancies is unproven and not medically necessary due to insufficient evidence of efficacy

Definitions

- **Added definition of:**
  - **Comprehensive Genomic Profiling (CGP)**
  - **Favorable Intermediate-Risk Prostate Cancer**
  - **Liquid Biopsy**
  - **Low-Risk Prostate Cancer**
  - **Predictive Molecular Markers**
  - **Prognostic Molecular Markers**
  - **Very-Low-Risk Prostate Cancer**
- **Updated definition of:**
  - **Comparative Genome Hybridization (CGH)**
  - **Chromosome Microarray Analysis (CMA)**
  - **Gene Expression Profiling (GEP)**

Applicable Codes

- **Added CPT codes 0016M, 0017M, 0120U, 0179U, 0204U, 0211U, 0239U, 0242U, 0244U, 0245U, 0250U, 0356U, 0362U, 0363U, 81529, 81546**
- **Removed CPT codes 0081U and 81545**
- **Revised description for CPT codes 0298U**
- **Added notation to indicate the following CPT codes are not on the State of Louisiana Medicaid Fee Schedule and therefore may not be covered by the State of Louisiana Medicaid Program: 0005U, 0011M, 0012M, 0013M, 0016M, 0017M, 0018U, 0019U, 0021U, 0022U, 0026U, 0036U, 0037U, 0045U, 0047U, 0048U, 0050U, 0069U, 0089U, 0090U, 0091U, 0113U, 0118U, 0120U, 0153U, 0171U, 0179U, 0204U, 0211U, 0239U, 0242U, 0244U, 0245U, 0250U, 0262U, 0285U, 0287U, 0288U, 0296U, 0297U, 0298U, 0299U, 0300U, 0306U, 0307U, 0313U, 0314U, 0315U, 0326U, 0329U, 0331U, 0332U, 0333U, 0334U, 0339U, 0340U, 0343U, 0356U, 0362U, 0363U, 81228, 81277, 81425, 81426, 81427, 81445, 81449, 81450, 81451, 81455, 81456, 81504, 81518, 81520, 81521, 81522, 81523, 81525, 81529, 81540, 81541, 81542, 81546, 81551, 81552, 81599, 86152, and 86153**
- **Removed ICD-10 diagnosis codes C91.40, C91.41, and C91.42**

Supporting Information

- **Updated *Description of Services, Clinical Evidence, FDA,* and *References* sections to reflect the most current information**
- **Archived previous policy version CS152LA.N**

Instructions for Use

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

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