MOLECULAR ONCOLOGY TESTING FOR CANCER DIAGNOSIS, PROGNOSIS, AND TREATMENT DECISIONS

Policy Number: LABORATORY 025.7 T2 **Effective Date**: January 1, 2019

Instructions for Use

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Related Policy

- Chemosensitivity and Chemoresistance Assays in Cancer

CONDITIONS OF COVERAGE

Applicable Lines of Business/Products	This policy applies to Oxford Commercial plan membership.
Benefit Type	General Benefits Package
Referral Required (Does not apply to non-gatekeeper products)	No
Authorization Required (Precertification always required for inpatient admission)	Yes[1]
Precertification with Medical Director Review Required	Yes[1]
Applicable Site(s) of Service (If site of service is not listed, Medical Director review is required)	Laboratory
Special Considerations	[1]Precertification with review by a Medical Director or their designee is required.

COVERAGE RATIONALE

Breast Cancer

The use of one of the following gene expression tests listed below is proven and medically necessary to make a treatment decision regarding adjuvant chemotherapy in females or males with non-metastatic breast cancer when all of the following criteria are met.

Use of more than one gene expression test for the same tumor in an individual with breast cancer is unproven and not medically necessary.

MammaPrint (also referred to as the "Amsterdam Signature" or "70-Gene Signature"), is proven and medically necessary to assess distant recurrence of disease in individuals with recently diagnosed non-metastatic breast cancer when ALL the following criteria are met:

- High clinical risk of recurrence based on at least one of the following criteria:
  - Lymph node positive (pN1-2); or
  - Tumor size greater than 2 cm; or
  - Poorly differentiated or undifferentiated histology (grade 3) AND tumor size greater than 1 cm; 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.

MammaPrint is unproven and not medically necessary for all other indications.

Oncotype Dx Breast, Prosigna PAM-50 Breast Cancer Prognostic Gene Signature Assay, EndoPredict and the Breast Cancer Index gene expression tests for intermediate and low risk breast cancer are proven and medically necessary to assess use of adjuvant chemotherapy in individuals with recently diagnosed non-metastatic breast cancer when all of the following criteria are met:
- Lymph node negative (pN0) or axillary lymph node micrometastasis less than 2mm (pN1mi); 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.

Oncotype Dx Breast, Prosigna PAM-50 Breast Cancer Prognostic Gene Signature Assay, EndoPredict, and the Breast Cancer Index are unproven and not medically necessary for all other indications.

Gene expression profiling assays for breast cancer treatment other than those previously described as covered are unproven and not medically necessary, including but not limited to:
- BluePrint (also referred to as "80-gene profile")
- Breast Cancer Gene Expression Ratio (also known as Theros H/I)
- BreastNext
- BreastOncPX
- BreastPRS
- Insight DX Breast Cancer Profile
- Mammostrat
- NexCourse Breast IHC4
- NuvoSelect eRx 200-Gene Assay
- Oncotype DX DCIS
- SYMPHONY Genomic Breast Cancer Profile
- TargetPrint
- TheraPrint
- The 41-gene signature assay
- The 76-gene "Rotterdam signature" assay

Thyroid Cancer

Molecular profiling of thyroid nodules (e.g., Afirma, ThyraMIR, Thyroseq) is proven and medically necessary when all of the following criteria are met:
- **Follicular pathology on fine needle aspiration is indeterminate**
- **The results of the test will be used for making decisions about further surgery**

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

Leukemia

Molecular profiling using chromosomal microarray analysis is proven and medically necessary for individuals with acute leukemia.

Lung Cancer

Molecular profiling of tumors using a multi-gene cancer panel of up to 50 genes is proven and medically necessary for individuals with metastatic non-small cell lung cancer (NSCLC).

Use of more than one gene multi-gene cancer panel for the same individual with non-small cell lung cancer is unproven and not medically necessary.

Molecular Profiling Tests for for Other Indications or Cancers

Whole Exome Sequencing (WES) and whole genomic sequencing (WGS) of tumors is unproven and not medically necessary for all indications.

Multi-gene cancer panels of greater than 50 genes are unproven and not medically necessary for all indications.

Molecularly profiling using gene expression profiling or multi-gene cancer panels is unproven and not medically necessary for all other indications, including but not limited to:

- **Bladder Cancer** (e.g., CytoScan® DX Assay)
- **Breast cancer other than those previously described as covered**
- **Cancers of Unknown Primary Site** (e.g., Response Dx, CancerTYPE ID, Rosetta Cancer Origin, ProOnc, SourceDX, Pathfinder TG)
- **Colorectal Cancer** (e.g., Oncotype DX Colon Cancer Assay, Colorectal Cancer DSA, GeneFx Colon, OncoDefender-CRC)
- **Leukemia other than those previously described as covered** (e.g., FoundationOne® Heme)
- **Melanoma** (e.g., Decision Dx – Melanoma, Decision Dx-UM)
- **Multiple myeloma** (e.g., MyPRS/MyPRS Plus)
- **Plasma detection of cell free DNA** (e.g., Guardant, Colonsentry)
- **Prostate cancer** (e.g., Oncotype DX Prostate Cancer Assay, TMPRSS2 fusion gene, Prolaris Prostate Cancer Test, Decipher Prostate Cancer Classifer)
- **Uveal melanoma** (e.g., Decision Dx-UM)

DEFINITIONS

Comparative Genome Hybridization (CGH): CGH is a technology that can be used for the detection of genomic copy number variations (CNVs). Tests can use a variety of probes or single nucleotide polymorphisms (SNPS) to provide copy number and gene differentiating information. All platforms share 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: A laboratory test that analyzes mRNA patterns to determine gene activity. (Kim et al. 2010)

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

Variant of Unknown Significance (VUS): A variation in a genetic sequence that has an unknown association with disease. It may also be called an unclassified variant. (Kamps, et al. 2017)

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)

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

APPLICABLE CODES

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

CPT Code	Description
0011M	Oncology, prostate cancer, mRNA expression assay of 12 genes (10 content and 2 housekeeping), RT-PCR test utilizing blood plasma and/or 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
0005U	Oncology (prostate) gene expression profile by real-time RT-PCR of 3 genes (ERG, PCA3, and SPDEF), urine, algorithm reported as risk score
0018U	Oncology (thyroid), microRNA profiling by RT-PCR of 10 microRNA sequences, utilizing fine needle aspirate, algorithm reported as a positive or negative result for moderate to high risk of malignancy
0019U	Oncology, RNA, gene expression by whole transcriptome sequencing, formalin-fixed paraffin embedded tissue or fresh frozen tissue, predictive algorithm reported as potential targets for therapeutic agents
0021U	Oncology (prostate), detection of 8 autoantibodies (ARF 6, NKX3-1, 5'-UTR-BMI1, CEP 164, 3'-UTR-Ropporin, Desmocollin, AURKAIP-1, CSNK2A2), multiplexed immunoassay and flow cytometry serum, algorithm reported as risk score
0022U	Targeted genomic sequence analysis panel, non-small cell lung neoplasia, DNA and RNA analysis, 23 genes, interrogation for sequence variants and rearrangements, reported as presence/absence of variants and associated therapy(ies) to consider
0026U	Oncology (thyroid), DNA and mRNA of 112 genes, next-generation sequencing, fine needle aspirate of thyroid nodule, algorithmic analysis reported as a categorical result ("Positive, high probability of malignancy" or "Negative, low probability of malignancy")
0036U	Exome (i.e., somatic mutations), paired formalin-fixed paraffin-embedded tumor tissue and normal specimen, sequence analyses
0037U	Targeted genomic sequence analysis, solid organ neoplasm, DNA analysis of 324 genes, interrogation for sequence variants, gene copy number amplifications, gene rearrangements, microsatellite instability and tumor mutational burden
0045U	Oncology (breast ductal carcinoma in situ), mRNA, gene expression profiling by real-time RT-PCR of 12 genes (7 content and 5 housekeeping), utilizing formalin-fixed paraffin-embedded tissue, algorithm reported as recurrence score
0047U	Oncology (prostate), mRNA, gene expression profiling by real-time RT-PCR of 17 genes (12 content and 5 housekeeping), utilizing formalin-fixed paraffin-embedded tissue, algorithm reported as a risk score
0048U	Oncology (solid organ neoplasia), DNA, targeted sequencing of protein-coding exons of 468 cancer-associated genes, including interrogation for somatic mutations and microsatellite instability, matched with normal specimens, utilizing formalin-fixed paraffin-embedded tumor tissue, report of clinically significant mutation(s)
0050U	Targeted genomic sequence analysis panel, acute myelogenous leukemia, DNA analysis, 194 genes, interrogation for sequence variants, copy number variants or rearrangements
0056U	Hematology (acute myelogenous leukemia), DNA, whole genome next-generation sequencing to detect gene rearrangement(s), blood or bone marrow, report of specific gene rearrangement(s)
0057U	Oncology (solid organ neoplasia), mRNA, gene expression profiling by massively parallel sequencing for analysis of 51 genes, utilizing formalin-fixed paraffin-embedded tissue, algorithm reported as a normalized percentile rank
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)

CPT Code	Description
81445	Targeted genomic sequence analysis panel, solid organ neoplasm, DNA analysis, and RNA analysis when performed, 5-50 genes (e.g., ALK, BRAF, CDKN2A, EGFR, ERBB2, KIT, KRAS, NRAS, MET, PDGFRA, PDGFRB, PGR, PIK3CA, PTEN, RET), interrogation for sequence variants and copy number variants or rearrangements, if performed
81450	Targeted genomic sequence analysis panel, hematolymphoid neoplasm or disorder, DNA analysis, and RNA analysis when performed, 5-50 genes (e.g., BRAF, CEBPA, DNMT3A, EZH2, FLT3, IDH1, IDH2, JAK2, KRAS, KIT, MLL, NRAS, NPM1, NOTCH1), interrogation for sequence variants, and copy number variants or rearrangements, or isoform expression or mRNA expression levels, if performed
81455	Targeted genomic sequence analysis panel, solid organ or hematolymphoid neoplasm, DNA analysis, and RNA analysis when performed, 51 or greater genes (e.g., ALK, BRAF, CDKN2A, CEBPA, DNMT3A, EGFR, ERBB2, EZH2, FLT3, IDH1, IDH2, JAK2, KIT, KRAS, MLL, NPM1, NRAS, MET, NOTCH1, PDGFRA, PDGFRB, PGR, PIK3CA, PTEN, RET), interrogation for sequence variants and copy number variants or rearrangements, if performed
81479	Unlisted molecular pathology procedure
81504	Oncology (tissue of origin), microarray gene expression profiling of > 2000 genes, utilizing formalin-fixed paraffin-embedded tissue, algorithm reported as tissue similarity scores
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
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
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
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
81599	Unlisted multianalyte assay with algorithmic analysis

DESCRIPTION OF SERVICES

Technologies used for molecular profiling of cancers vary, and can include, but are not limited to,tests that evaluate variations in the genes, such as chromosome microarray and next generation sequencing, as well as others that assess the gene products, such as gene expression arrays and microRNA analysis. The number of genes evaluated can range from a single gene to the whole exome or genome of a tumor. In some cases, expression patterns of defined genes are combined in a defined manner to provide an expression signature, a score, or a classifier for potential diagnosis and or prognosis of disease or to predict impact of intervention. Results of molecular profiling may assist individuals and healthcare providers with determining prognosis and selection of more effective and targeted cancer therapies. (Chantrill et al., 2015)

CLINICAL EVIDENCE

Breast Cancer

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

The National Comprehensive Cancer Network (NCCN) clinical guidelines for breast cancer (2017) state that the use of DNA microarray technologies to characterize breast cancer has allowed for development of classifications of breast cancer by gene expression profile. Five major subtypes of breast cancer have been identified by DNA microarray gene expression profiling. In retrospective analyses, these gene expression subtypes are associated with differing relapse-free survival and overall survival (OS).

Oncotype Dx® Breast

Oncotype Dx Breast (Oncotype DX; Genomic Health, Redwood City, CA) s a test that analyzes the expression of a panel of 21 genes within a tumor to determine a "Recurrence Score" which may correspond to a likelihood of breast cancer recurrence within 10 years. The test was initially developed for women with early-stage invasive breast cancer with ER+ cancers that are lymph node-negative, and subsequently evidence was gathered on individuals with up to 3 ipsilateral nodes positive. These individuals are typically treated with anti-hormonal therapy, such as tamoxifen or aromatase inhibitors, and Oncotype Dx® can help determine if chemotherapy should be added to the treatment regimen. [Evaluation of Genomic Applications in Practice and Prevention (EGAPP) Working Group, 2015]

The Evaluation of Genomic Applications in Practice and Prevention (EGAPP) working group (2016) found insufficient evidence to recommend for or against the use of Oncotype DX testing to guide chemotherapy treatment decisions in women with hormone receptor-positive, lymph node-negative, or lymph node-positive early breast cancer who are receiving endocrine therapy. This recommendation statement updates a 2009 EGAPP statement on the use of gene expression profiling tests in breast cancer. Evidence of clinical validity for Oncotype DX was confirmed by EGAPP as adequate. With regard to clinical utility, although there was evidence from prospective retrospective studies that the Oncotype DX test predicts benefit from chemotherapy, and there was adequate evidence that the use of Oncotype DX gene expression profiling in clinical practice changes treatment decisions regarding chemotherapy, EGAPP found no direct evidence that the use of Oncotype DX testing leads to improved clinical outcomes.

Wolmark et al. (2016) assess the utility for a 21 gene recurrence score (RS) in predicting distance recurrence (>5 years) in stages I and II breast cancer in high and low expressing ESR1 groups within a cohort of 3,060 patients from the National Surgical Adjuvant Breast and Bowel project, all of whom had undergone tamoxifen therapy. Overall, the authors found that RS consistently predicted distant recurrence; low RS had a low risk of distant recurrence. In a subgroup analysis, it was noted that individuals with a low RS and 1-3 node positives, the risk of distant recurrence was 7.9%. In those with 4 or more nodes positive, the risk of distant recurrence was 16.7%.

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

Oncotype Dx DCIS

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

Prosigna® Breast Cancer Prognostic Gene Signature Assay

The Prosigna® (NanoString Technologies (Seattle, WA) breast cancer prognostic assay provides a risk category and numerical score to assess an individual's risk of distant recurrence of disease at 10 years in postmenopausal women

with node-negative (Stage I or II) or node-positive (Stage II), hormone receptor-positive breast cancer. The Prosigna assay measures expression levels of 50 genes using formalin-fixed paraffin-embedded (FFPE) breast tumor tissue diagnosed as invasive breast carcinoma. The assay is not intended for individuals with 4 or more positive nodes. (Gnant et al., 2013; Parker et al., 2009)

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

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

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

EndoPredict

EndoPredict (Sividon Diagnostics [acquired by Myriad (Salt Lake City, UT) in 2016] is a 12-gene real-time RT-PCR that includes eight disease-relevant genes BIRC5, UBE2C, DHCR7, RBBP8, IL6ST, AZGP1, MGP and STC2 are compared to three RNA normalization genes (CALM2, OAZ1 and RPL37A) and to one DNA reference gene (HBB).

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

Breast Cancer Index (BCI)

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

Sestak et al. (2016) conducted a retrospective analysis to examine cross-straification between Breast Cancer Index (BCI) and the OncotypeDX Recurrence Score (RS) to directly compare their prognostic accuracy at the individual patient level. Six hundred and sixty-five patients with hormone receptor-positive (HR^+) and lymph node-negative disease were included in this retrospective analysis. The authors concluded that BCI demonstrated increased prognostic accuracy versus RS. Notably, BCI identified subsets of RS low and RS intermediate risk patients with significant and clinically relevant rates of DR. These results indicate that additional subsets of women with HR^+, lymph node-negative breast cancer identified by BCI may be suitable candidates for adjuvant chemotherapy or extended endocrine therapy.

Professional Societies

American Society of Clinical Oncology (ASCO)

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

In their 2016 evidence-based guideline on the use of biomarkers to guide decisions on adjuvant systemic therapy for women with early-stage invasive breast cancer, ASCO (Harris et al., 2016a; Harris et al., 2016b) found sufficient evidence of clinical utility for the biomarker assays Onco*type* DX, EndoPredict, PAM50, Breast Cancer Index, and urokinase plasminogen activator and plasminogen activator inhibitor type 1 in specific subgroups of breast cancer. No biomarker except for estrogen receptor, progesterone receptor, and human epidermal growth factor receptor 2 was found to guide choices of specific treatment regimens. Treatment decisions should also consider disease stage, comorbidities, and patient preferences.

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

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

American College of Radiology (ACR)

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

Melanoma

Cutaneous Melanoma

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.

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

Wiesner et al. (2016) provided a review on the diagnostic, prognostic, and therapeutic value of understanding genomic alterations in spitzoid tumors. Spitzoid tumors are composed of large spindle shaped or epithelioid melanocytes, and are biologically distinct from melanocytic naevi and melanoma. Naevi and melanoma may have BRAF, NRAS mutations or inactivation of NF-1, Spitz tumors often have genomic rearrangements or HRAS mutation, or inactivation of BAP1. The number of genomic alterations correlates with the degree of abnormal histology and CGH analysis or FISH can accurately classify benign and malignant Spitz tumors. However, 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 a 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 has positive sentinel lymph node biopsy, 1 of which also had distant metastasis. Also evaluated were 8 patients with Spitz nevi, and 3 patients with melanoma (2 spitzoid, 1 superficial spreading). Chromosomal aberrations were found in 7 patients with ambivalent melanocytes, and there was no difference between the positive and negative lymph node biopsy groups. One had a fatal outcome. Chromosome abnormalities were also found in 2 spitzoid melanomas, and 1 conventional melanoma. The majority of aberrations found in the ambivalent group were not the ones commonly found in melanomas, suggesting that this may be a unique clinical entity. FISH failed to detect one spitzoid melanoma, 1 fatal metastatic case, and the other chromosomally aberrant ambivalent cases. It was positive in 1 spitzoid melanoma and 1 conventional. Overall the authors concluded that CGH may offer better diagnostic aid with better sensitivity and specificity than FISH in atypical Spitz tumors.

Professional Societies

The National Comprehensive Cancer Network (NCCN)

NCCN (2016) clinical practice guidelines for melanoma note that while there is interest in the newer prognostic molecular techniques such as gene expression profiling to differentiate benign from malignant neoplasms, or melanoma at low versus high risk for metastasis, routine (baseline) testing of primary cutaneous melanoma (before or following SLNB) is not recommended outside of clinical study (trial).

Uveal Melanoma

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

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

Minca et al. (2014) noted that monosomy 3 and MYC amplification at 8q24 are strong prognosticators of outcomes in uveal melanoma (UVM), and is commonly detected by FISH. They hypothesized that CMA would be an alternative to FISH and have advantages in identifying loss of heterozygosity, partial chromosome loss and other aberrations that FISH can't detect. They analyzed CMA using SNP+CGH (Roche-NimbleGen OncoChip) on enucleations from formalin-

fixed paraffin-embedded tissue (FFPET) for 34 patients and/or frozen tissue (FZT) for 41 patients. CMA was successful in 30 of 30 of 34 FFPET and all 41 FZT. In 27 paired FFPET/FZT samples 96% were concordant for at least 4 of 6 major chromosome abnormalities, and 93% were concordant for one (- chromosome 3). CMA was concordant with FISH in 90% of FFPET and 93% of FZT. Partial -3q was detected in two FISH negative cases, and whole chromosome LOH for 3, 4 and 6 in one case. Results of UVM SNP+CGH genotyping were significantly correlated with clinical outcome and reliably predicted metastasis, time to progression, and survival. The authors concluded that SNP+CGH is a practical method for UVM prognostication, and provides additional data with relevance to biology, diagnosis and prognosis.

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

Professional Societies

American Academy of Dermatology (AAD)

The AAD does not address molecular testing in their guidelines at any point, and states in general that baseline laboratory tests are generally not recommended in 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)

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)

The National Comprehensive Cancer Network (NCCN)

NCCN introduced guidelines in 2018 for the staging and management of uveal melanoma. While they note that tumor specimens may be sent for chromosome analysis and/or gene expression profiling, they note that biopsy is usually not necessary for initial diagnosis or to make a treatment selections and does not impact patient outcomes.

Cancers of Unknown Primary Site

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

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 Comprehensive Cancer Network (NCCN)

National Comprehensive Cancer Network (NCCN) clinical practice guidelines for occult primary (cancer of unknown primary site) state that while there is diagnostic benefit of gene expression profiling (GEP) assays, a clinical benefit has not been demonstrated. Consequently, the panel does not recommend tumor sequencing and gene signature profiling for the identification of tissue of origin as standard management in the diagnostic workup of patients with occult primary tumors. In addition, the NCCN suggests that pathologists and oncologists collaborate on the judicious use of these modalities on a case-by-case basis, with the best individualized patient outcome in mind. (NCCN, 2016)

Colorectal Cancer (CRC)

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

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

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

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

In a validation study of the 12-gene colon cancer recurrence score in NSABP C-07 as a predictor of recurrence in patients with stage II and III colon cancer treated with fluorouracil and leucovorin (FU/LV) and FU/LV plus oxaliplatin, Yothers et al. (2013). Recurrence Score was assessed in 892 fixed, paraffin-embedded tumor specimens (randomly selected 50% of patients with tissue). Data were analyzed by Cox regression adjusting for stage and treatment. Based on the results, the authors concluded that 12-gene Recurrence Score predicts recurrence risk in stage II and stage III colon cancer and provides additional information beyond conventional clinical and pathologic factors. Incorporating Recurrence Score into the clinical context may better inform adjuvant therapy decisions in stage III as well as stage II colon cancer.

Professional Societies

The National Comprehensive Cancer Network (NCCN)

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, 2018)

Multiple Myeloma

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.

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.

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.

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.

Professional Societies

The National Comprehensive Cancer Network (NCCN)

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, 2016)

Leukemia

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. 27 patients with hematological malignancies were evaluated by chromosome analysis, FISH and CGH or CGH+SNP arrays. Nearly 90% of chromosome abnormalities found in the patients were also identified by microarray. Of 183 CNVs found, 52% were additional anomalies that were not found by routine cytogenetics or FISH. 65% were <10 Mb in size. Balanced rearrangements were not found by microarray, but of 19 rearrangements that appeared "balanced" by routine cytogenetics, 7 had alterations found by microarray at the breakpoints. The authors concluded that CGH provided clinicians with advantages in identification of cryptic imbalances and clonal abnormalities in non-dividing cells with poor chromosome morphology and therefore had potential to be integrated as a patient management tool.

Laurie et al. (2015) compared the SNP array results of 278 symptomatic CLL patients with >50,000 subjects from the GENEVA consortium of genome wide association studies, which analyzed people with a range of medical conditions and healthy controls. The CLL patients were also analyzed by FISH to determine performance and concordance between the SNP array and FISH. When a parameter of 20% abnormal cells was used as a cutoff, the concordance rate between the SNP array and FISH was 98.9%. The array found 8.4% of cases with UPD which cannot be detected by FISH. In 214 CLL patients with SNP results, 1112 genetic anomalies were found, of which 628 were considered acquired. This was a higher percentage and anomalies were unique in the CLL group when compared to the GENEVA cohort and suggests that late stage CLL has recurrent acquired anomalies that do not occur in precursor conditions or in the general population. The clinical significance of this finding is not clear, however, SNP based array was demonstrated to be a valid analysis tool.

Koh et al. (2014) utilized a CGH+SNP array platform to study the presence of CNVs and LOH in 15 children with acute myeloid leukemia (AML) and 3 with myelodysplastic syndrome (MDS). Cytogenetic analysis revealed CNV in 11

regions in 8 patients. SNP+CGH found 14 CNV in 9 patients, and cryptic LOHs in 3 of 5 patients with normal cytogenetics. Overall, 9 patients were found to have abnormalities not detected by routine cytogenetics. 3 patients with AML and terminal LOH of >10Mb had significantly inferior relapse-free survival time, suggesting that SNP+CGH testing can provide additional prognostic information.

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

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

Boultwood et al. (2010) used a SNP array to analyze 41 chronic myeloid leukemia (CML) patients using 53 bone marrow or blood sample. 32 were in chronic phase and 21 were in blast crises. The samples were analyzed for uniparental disomy (UPD) and copy number variants, with quality control comparisons with 100 healthy controls of different ethnicities for SNP array hybridization intensities, and 45 healthy controls as a reference set. Across the samples 44 regions of UPD were identified, with chromosome 8 having the highest frequency. 10 regions of copy number variation 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 (ie, 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.

Prostate Cancer

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.

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.

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 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.

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.

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

clinical recurrence. The authors conclude that Decipher improves the ability to predict clinical recurrence in prostate cancer and adds precision to conventional pathological prognostic measures. Long-term studies are needed to validate these results.

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

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 identfied, 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.

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.

Professional Societies

American Urological Association (AUA)

In a clinical practice guideline on early detection of prostate cancer (Carter et al., 2013; reviewed and confirmed 2015) based on a systematic review and meta-analysis, the AUA notes that an improved understanding of the interaction between inherited risk alleles and the environment (lifestyle choices) could provide a potential means of prevention. Future studies of the genetic and epigenetic basis of disease development and progression may provide biomarkers and/or panels of biomarkers with improved specificity when compared to PSA. When available, risk assessment tools combining multiple predictors will need to be evaluated in carefully designed trials to be generalizable to the population in which they would be used.

American Society of Clinical Oncology (ASCO)

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)

The National Comprehensive Cancer Network (NCCN)

NCCN clinical practice guidelines (2018) for prosate cancer state that molecular profiling of biopsies may be considered in men with low and favorable intermediate risk prostate cancer and a life expectancy greater than or equal to ten years to help guide decision-making on treatment. However, NCCN cautions that these tests 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, 2018)

Lung Cancer

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

Professional Societies

American College of Chest Physicians (ACCP)

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

American Society of Clinical Oncology (ASCO)

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

- Physicians may use molecular biomarker testing in tumors with:
  - An adenocarcinoma component;
  - 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.

The 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 drugable 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 and ThyroSeq. 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 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 surgey; the majority (88%) being performed within 2 years. According to the authors, this represents a reduction in thyroid surgeries compared to patients that did not have a GEC performed on suspicious lesions. Limitations of this study are small patient population and non-randomization of patients.

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

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

The National Comprehensive Cancer Network (NCCN) guidelines for Thyroid Carcinoma (NCCN, 2017) 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 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 nondiagnosticcytology 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. 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)

NCCN guidelines for Thyroid Carcinoma (NCCN, 2017) 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.

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 for the following:

- Acute myeloid leukemia (Port et al., 2014; Link et al., 2012)
- Adrenocortical cancer (Zheng et al., 2016; Ross et al., 2014a)
- Breast cancer (Ganesan et al., 2014; Wheler et al., 2014)
- 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)
- Non-melanoma skin cancers
- Pancreatic cancer (Zhou et al., 2017; Chmielecki et al., 2014; Chantrill et al., 2015)
- Urothelial carcinoma/urinary bladder adenocarcinoma (Roy et al., 2017; Ross et al., 2014b; Millis et al., 2015)

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

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 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.

U.S. FOOD AND DRUG ADMINISTRATION (FDA)

Laboratories that perform genetic tests are regulated under the Clinical Laboratory Improvement Amendments (CLIA) Act of 1988. More information is available at: https://www.fda.gov/medicaldevices/deviceregulationandguidance/ivdregulatoryassistance/ucm124105.htm. (Accessed September 12, 2018)

A search of the FDA website identified an approval (K042279) for the Affymetrix Genechip Microarray Instrumentation System on December 23, 2004. See the following website for more information: http://www.accessdata.fda.gov/cdrh_docs/pdf4/K042279.pdf. (Accessed September 12, 2018)

The CytoScan® DX Assay (Affymetrix, Inc.) was cleared for marketing under the FDA's 510(k) process in January 2014. The FDA classifies the devices as a Type II postnatal chromosomal copy number variation detection system. According to documents filed with FDA, CytoScan Dx Assay is a qualitative assay intended for the postnatal detection of copy number variations (CNV) in genomic DNA obtained from peripheral whole blood in patients referred for chromosomal testing based on clinical presentation. CytoScan Dx Assay is intended for the detection of CNVs associated with developmental delay, intellectual disability, congenital anomalies, or dysmorphic features. The CytoScan DX Assay is a microarray that works with Affymetrix's existing GeneChip technology platform to perform comparative whole-genome hybridization. This device is not intended to be used for standalone diagnostic purposes, preimplantation or prenatal testing or screening, population screening, or for the detection of, or screening for, acquired or somatic genetic aberrations. The FDA's review of the CytoScan Dx Assay included an analytic evaluation of the test's ability to accurately detect numerous chromosomal variations of different types, sizes, and genome locations when compared with several analytically validated test methods. FDA found that the CytoScan Dx Assay could analyze a patient's entire genome and adequately detect chromosome variations in regions of the genome associated with intellectual and developmental disabilities. See the following websites for more information:

- https://www.accessdata.fda.gov/cdrh_docs/pdf13/K130313.pdf
- http://www.accessdata.fda.gov/cdrh_docs/reviews/k130313.pdf

(Accessed September 12, 2018)

REFERENCES

The foregoing Oxford policy has been adapted from an existing UnitedHealthcare national policy that was researched, developed and approved by UnitedHealthcare Medical Technology Assessment Committee. [*2019T0588G*]

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

Albain KS, Barlow WE, Shak S et al. Prognostic and predictive value of the 21-gene recurrence score assay in postmenopausal women with node-positive, oestrogen-receptor-positive breast cancer on chemotherapy: a retrospective analysis of a randomised trial. Lancet Oncol 2010; 11: 55–65.

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

Alexander EK, Schorr M, Klopper J, et al. Multicenter clinical experience with the Afirma gene expression classifier. J Clin Endocrinol Metab. 2014;99(1):119–125.

Ali SM, Sanford EM, Klempner SJ, et al. Prospective comprehensive genomic profiling of advanced gastric carcinoma cases reveals frequent clinically relevant genomic alterations and new routes for targeted therapies. Oncologist. 2015;20(5):499-507.

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

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

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

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

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

Bichakjian CK, Halpern AC, Johnson TM, et al. Guidelines of care for the management of primary cutaneous melanoma. American Academy of Dermatology. J Am Acad Dermatol. 2011 Nov;65(5):1032-47.

Boultwood J., Perry J., Zaman R., et al. High-density single nucleotide polymorphism array analysis and ASXL1 gene mutation screening in chronic myeloid leukemia during disease progression. Leukemia. 2010;24(6):1139–1145.

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

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

Buus R, Sestak I, Kronenwett R, et al. Comparison of EndoPredict and EPclin with oncotype dx recurrence score for prediction of risk of distant recurrence after endocrine therapy. J Natl Cancer Inst. 2016 Jul 10;108(11).

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

Carter HB, Albertsen PC, Barry MJ, et al. American Urological Association (AUA). Early detection of prostate cancer. Published 2013; reviewed and validity confirmed 2015.

Chantrill LA, Nagrial AM, Watson C, et al. Australian Pancreatic Cancer Genome Initiative (APGI) and the Individualized Molecular Pancreatic Cancer Therapy (IMPaCT) Trial Management Committee of the Australasian Gastrointestinal Trials Group (AGITG). Precision medicine for advanced pancreas cancer: the Individualized Molecular Pancreatic Cancer Therapy (IMPaCT) Trial. Clin Cancer Res. 2015 May 1;21(9):2029-37.

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

Chmielecki J, Hutchinson KE, Frampton GM, et al. Comprehensive genomic profiling of pancreatic acinar cell carcinomas identifies recurrent RAF fusions and frequent inactivation of DNA repair genes. Cancer Discov. 2014 Dec;4(12):1398-405.

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

Chung CH, Guthrie VB, Masica DL, et al. Genomic alterations in head and neck squamous cell carcinoma determined by cancer gene-targeted sequencing. Ann Oncol. 2015 Feb 23. pii: mdv109.

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

Crawford ED, Scholz MC, Kar AJ, et al. Cell cycle progression score and treatment decisions in prostate cancer: results from an ongoing registry. Curr Med Res Opin. 2014 Jun;30(6):1025-31.

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

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

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

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

Duick DS, Klopper J, Diggins, J et al. The impact of benign gene expression classifier test results on the endocrinologist/patient decision to operate on patients with thyroid nodules with indeterminate fine-needle aspiration cytopathology. Thyroid. 2012 Oct; 22(10): 996–1001.

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

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

Evans AG, Ahmad A, Burack WR, Iqbal MA. "Combined comparative genomic hybridization and single-nucleotide polymorphism array detects cryptic chromosomal lesions in both myelodysplastic syndromes and cytopenias of undetermined significance." Modern pathology: an official journal of the United States and Canadian Academy of Pathology, Inc. 2016 Oct 0; 29(10):1183-99.

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

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

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

Gatalica Z, Millis SZ, Vranic S, et al. Comprehensive tumor profiling identifies numerous biomarkers of drug response in cancers of unknown primary site: analysis of 1806 cases. Oncotarget. 2014 Dec 15;5(23):12440-7.

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

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

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

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

Hagenkord JM, Monzon FA, Kash SF, et al. Array-based karyotyping for prognostic assessment in chronic lymphocytic leukemia: Performance comparison of Affymetrix 10K2.0, 250K Nsp, and SNP6.0 Arrays. The Journal of Molecular Diagnostics : JMD. 2010;12(2):184-196.

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

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

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

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

Hirshfield KM, Tolkunov D, Zhong H′ et al. Clinical actionability of comprehensive genomic profiling for management of rare or refractory cancers. Oncologist. 2016 Aug 26. pii: theoncologist. 2016-0049. [Epub ahead of print]

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

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

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

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

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

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

Kim K, Zakharkin SO, Allison DB. Expectations, validity, and reality in gene expression profiling. Journal of clinical epidemiology. 2010;63(9):950-959.

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

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

Koh KN, Lee JO, Seo EJ, et al. Clinical significance of previously cryptic copy number alterations and loss of heterozygosity in pediatric acute myeloid leukemia and Myelodysplastic Syndrome determined using combined array comparative genomic hybridization plus single-nucleotide polymorphism microarray analyses. Journal of Korean Medical Science. 2014;29(7):926-933.

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

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

Kutzner H, Metzler G, Argenyi Z, et al. Histological and genetic evidence for a variant of superficial spreading melanoma composed predominantly of large nests. Mod Pathol. 2012;25:838–45.

Labourier E, Shifrin A, Busseniers AE, et al. Molecular testing for miRNA, mRNA, and DNA on fine-needle aspiration improves the preoperative diagnosis of thyroid nodules with indeterminate Cytology. J Clin Endocrinol Metab 2015; 100: 2743–2750.

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

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

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

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

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

Merker JD, Oxnard GR, Compton C, et al. Circulating tumor DNA analysis in patients with cancer: American Society of Clinical Oncology and College of American Pathologists Joint Review. J Clin Oncol. 2018 Mar 5:JCO2017768671.

Millis SZ, Bryant D, Basu G, et al. Molecular profiling of infiltrating urothelial carcinoma of bladder and nonbladder origin. Clin Genitourin Cancer. 2015 Feb;13(1):e37-49.

Minca EC, Tubbs RR, Portier BP, et al. Genomic microarray analysis on formalin-fixed paraffin-embedded material for uveal melanoma prognostication. Cancer Genet. 2014;207:306–315.

Miura JT, Johnston FM, Thomas J, et al. Molecular profiling in gastric cancer: examining potential targets for chemotherapy. J Surg Oncol. 2014 Sep;110(3):302-6.

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

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

National Comprehensive Cancer Network (NCCN). Clinical Practice Guidelines in Oncology. Breast cancer. Version 2.2017 – April 6, 2017.

National Comprehensive Cancer Network (NCCN). Clinical Practice Guidelines in Oncology. Colon cancer. Version 2.2018 – March 14, 2018.

National Comprehensive Cancer Network (NCCN). Clinical Practice Guidelines in Oncology. Melanoma. Version 1. 2017 – November 10, 2016.

National Comprehensive Cancer Network (NCCN). Clinical Practice Guidelines in Oncology. Multiple myeloma. Version 3.2017 – November 28, 2016.

National Comprehensive Cancer Network (NCCN). Clinical Practice Guidelines in Oncology. Non-small cell lung cancer. Version 7.2017 – June 7, 2017.

National Comprehensive Cancer Network (NCCN). Clinical Practice Guidelines in Oncology. Occult primary [cancer of unknown primary (CUP)]. V2.2017 – October 10, 2017.

National Comprehensive Cancer Network (NCCN). Clinical Practice Guidelines in Oncology. Prostate cancer. Version 2.2018 – February 14, 2018.

National Comprehensive Cancer Network (NCCN). Clinical Practice Guidelines in Oncology. Rectal cancer. Version 2.2018 – March 14, 2018.

National Comprehensive Cancer Network (NCCN). Clinical Practice Guidelines in Oncology. Thyroid Carcinoma V2.2017 – May 5, 2017.

National Comprehensive Cancer Network (NCCN). Clinical Practice Guidelines in Oncology. Uveal melanoma. Version 1.2018 – March 15, 2018.

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

Nikiforova MN, Mercurio S, Wald A, et al. Analytical performance of the ThyroSeq version 3 genomic classifier for cancer diagnosis in thyroid nodules. Cancer 2018.

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

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

Onken MD, Worley LA, Char DH, et al. Collaborative Ocular Oncology Group report number 1: prospective validation of a multi-gene prognostic assay in uveal melanoma. Ophthalmology. 2012 Aug;119(8):1596-603.

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

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

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

Partyka KL Randolph ML, Lawrence KA, et al. Utilization of direct smears of thyroid fine-needle aspirates for ancillary molecular testing: A comparison of two proprietary testing platforms. Diagn Cytopathol. 2018 Apr;46(4):320-325.

PDQ Adult Treatment Editorial Board. Intraocular (Uveal) Melanoma Treatment (PDQ®): Health Professional Version. 2018 Feb 8.

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

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

Port M, Böttcher M, Thol F, et al. Prognostic significance of FLT3 internal tandem duplication, nucleophosmin 1, and CEBPA gene mutations for acute myeloid leukemia patients with normal karyotype and younger than 60 years: a systematic review and meta-analysis. Ann Hematol. 2014 Aug;93(8):1279-86.

Puiggros A, Puigdecanet E, Salido M, et al. Genomic arrays in chronic lymphocytic leukemia routine clinical practice: are we ready to substitute conventional cytogenetics and fluorescence in situ hybridization techniques? Leuk Lymphoma. 2013;54:986–995.

Raskin L, Ludgate M, Iyer RK et al. Copy number variations and clinical outcome in Atypical Spitz Tumors. The American Journal of Surgical Pathology: 2011 35(2) : p 243–252.

Rodriguez-Rodriguez L, Hirshfield KM, Rojas V, et al. Use of comprehensive genomic profiling to direct point-of-care management of patients with gynecologic cancers. Gynecol Oncol. 2016;141(1):2-9.

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

Ross JS, Wang K, Al-Rohil RN, et al. Advanced urothelial carcinoma: next-generation sequencing reveals diverse genomic alterations and targets of therapy. Mod Pathol. 2014b Feb;27(2):271-80.

Ross JS, Wang K, Gay L et al. Comprehensive genomic profiling of carcinoma of unknown primary site: new routes to targeted therapies. JAMA Oncol. 2015;1(1):40-49.

Ross JS, Wang K, Rand JV, et al. Next-generation sequencing of adrenocortical carcinoma reveals new routes to targeted therapies. J Clin Pathol. 2014a Nov;67(11):968-73.

Roy S, Pradhan D, Ernst WL, et al. Next-generation sequencing-based molecular characterization of primary urinary bladder adenocarcinoma. Mod Pathol. 2017 May 26. [Epub ahead of print]

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

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

Shore N, Concepcion R, Saltzstein D, et al. Clinical utility of a biopsy-based cell cycle gene expression assay in localized prostate cancer. Curr Med Res Opin. 2014 Apr;30(4):547-53.

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

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

Song Q, Chu Y, Yao Y, et al. Identify latent chromosomal aberrations relevant to myelodysplastic syndromes. Scientific Reports. 2017b;7:10354.

Thiel A, Beier M, Ingenhag D, et al. Comprehensive array CGH of normal karyotype myelodysplastic syndromes reveals hidden recurrent and individual genomic copy number alterations with prognostic relevance. Leukemia 2011; 25: 387–399.

Tiu RV, Gondek LP, O'Keefe CL, et al. Prognostic impact of SNP array karyotyping in myelodysplastic syndromes and related myeloid malignancies. Blood. 2011;117(17):4552-4560.

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

U.S. National Library of Medicine. What are whole exome sequencing and whole genome sequencing? Genetics Home Reference. October 2017A. Available at: https://ghr.nlm.nih.gov/primer/genomicresearch/sequencing.

U.S. National Library of Medicine. Do all gene mutations affect health and development? Genetics Home Reference. October 2017B. Available at: https://ghr.nlm.nih.gov/primer/genomicresearch/sequencing.

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

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

Vignot S, Lefebvre C, Frampton GM, et al. Comparative analysis of primary tumour and matched metastases in colorectal cancer patients: Evaluation of concordance between genomic and transcriptional profiles. Eur J Cancer. 2015 May;51(7):791-9.

Wang K, McDermott JD, Schrock AB, et al. Comprehensive genomic profiling of salivary mucoepidermoid carcinomas reveals frequent BAP1, PIK3CA, and other actionable genomic alterations. Ann Oncol. 2017 Apr 1;28(4):748-753.

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

West AC, Tang K, Tye H, et al. Identification of a TLR2-regulated gene signature associated with tumor cell growth in gastric cancer. Oncogene. 2017 May 8. [Epub ahead of print]

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

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

Wiesner T, Kutzner H, Cerroni L, et al. Genomic aberrations in spitzoid tumours and their implications for diagnosis, prognosis and therapy. Pathology. 2016;48(2):113-131.

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

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

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

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

Yang C, Zou K, Yuan Z, Guo T, Xiong B. Prognostic value of circulating tumor cells detected with the CellSearch System in patients with gastric cancer: evidence from a meta-analysis. OncoTargets and therapy. 2018;11:1013-1023.

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

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

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

Zhang M and Lin O Molecular Testing of Thyroid Nodules: A Review of Current Available Tests for Fine-Needle Aspiration Specimens. Archives of Pathology & Laboratory Medicine: December 2016, Vol. 140, No. 12, pp. 1338-1344.

Zheng S, Cherniack AD, Dewal N, et al. Comprehensive pan-genomic characterization of adrenocortical carcinoma. Cancer Cell. 2016 May 9; 29(5): 723–736.

Zhou H, Chen Q, Tan W, et al. Integrated clinicopathological features and gene microarray analysis of pancreatic neuroendocrine tumors. Gene. 2017 May 4. pii: S0378-1119(17)30332-3. [Epub ahead of print]

POLICY HISTORY/REVISION INFORMATION

Date	Action/Description

01/01/2019	• Reorganized policy template:
	  ○ Simplified and relocated *Instructions for Use*
	  ○ Removed *Benefit Considerations* section
	• Updated coverage rationale; modified language to clarify the listed services are:
	  ○ Proven ***and*** medically necessary (as described)
	  ○ Unproven ***and*** not medically necessary (as described)
	• Updated list of applicable CPT codes; added 81425, 81426, 81427, 81479, and 81518* *(*annual code edit)*
	• Archived previous policy version LABORATORY 025.6 T2

INSTRUCTIONS FOR USE

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

The term Oxford includes Oxford Health Plans, LLC and all of its subsidiaries as appropriate for these policies. Unless otherwise stated, Oxford policies do not apply to Medicare Advantage members.

UnitedHealthcare may also use tools developed by third parties, such as the MCG™ Care Guidelines, to assist us in administering health benefits. UnitedHealthcare Oxford Clinical 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.