Molecular Pathology/Genetic Testing Reported with Unlisted Codes

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Instructions for Use

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Coverage Rationale

Overview

When reporting the service performed, providers should select the specific code that accurately identifies the service performed. However, some services may not have a specific code; therefore, when reporting for these services, unlisted codes are designated. Unlisted codes provide the means of reporting and tracking services until a more specific code is established. If no such specific code exists, reporting the service using the appropriate unlisted service code would be appropriate. Unlisted codes should be reported only if no other specific codes adequately describe the procedure or service. When reporting a laboratory test(s) using an unlisted code, the specific name of the laboratory test(s) and/or a short descriptor of the test(s) must be included.

Based on the Centers for Medicare & Medicaid Services (CMS) Program Integrity Manual (100-08), this policy addresses the circumstances under which the item or service is reasonable and necessary under the Social Security Act, §1862(a)(1)(A). For laboratory services, a service can be reasonable and necessary if the service is safe and effective; not experimental or investigational (exception: routine costs of qualifying clinical trial services which meet the requirements of the Clinical Trials NCD and are reasonable and necessary); and appropriate, including the duration and frequency that is appropriate for the item or service, in terms of whether it is furnished in accordance with accepted standards of medical practice for the diagnosis or treatment of the patient's condition or to improve the function of a malformed body member; furnished in a setting appropriate to the patient's medical needs and condition; ordered and furnished by qualified personnel; one that meets, but does not exceed, the patient's medical need; and is at least as beneficial as an existing and available medically appropriate alternative.

CMS National Coverage Determinations (NCDs)

Medicare has an NCD 90.2 Next Generation Sequencing (NGS), and compliance is required where applicable.

CMS Local Coverage Determinations (LCDs) and Articles

Local Coverage Determinations (LCDs)/Local Coverage Articles (LCAs) exist, and compliance with these policies is required where applicable. For specific LCDs/LCAs, refer to the Medicare Coverage Database and/or the table in this policy under CMS Related Documents.

For coverage guidelines for states/territories with no LCDs/LCAs, refer to the coverage rationale below.

Covered Indications for CPT Code 81479

Specific diagnosis criteria for reasonable and necessary services can be found in the Applicable Codes section.

Biomarkers for Oncology

- MyPRS Genetic Expression Profile Testing is reasonable and necessary only after the initial diagnosis of multiple myeloma has been made and will be available to be used in the stratification of therapeutic interventions. It would be inappropriate to use this test as a diagnostic tool or as a monitoring device of ongoing therapy. Other testing is available for this function.
- Rosetta Cancer Origin Test™ Molecular testing, using the Rosetta Cancer Origin Test™ (PROG), is reasonable and necessary in the pathologic diagnoses of cancer of unknown primary (CUP) when a conventional surgical pathology/imaging work-up is unable to identify a primary neoplastic site. Other applications of this technology are not reasonable and necessary and are investigational in the use of diagnosis of specific tumor types such as NSCLC and renal cancers.
- RosettaGX Reveal thyroid MicroRNA test is an assay used for the classification of indeterminate thyroid nodules.
- Uveal Melanoma GNA11
- CIMP
- PTEN
- AKT1
- RB1
- MLL/AF4
- DEK/CAN
- TET2
- CALR
- CSF3R
- TSC2
- FGFR1
- MTOR
- BIRC3
- FBXW7
- JAK1
- JAK3
- STAT5B

Pathfinder TG®/PancraGEN

PathfinderTG® is reasonable and necessary when selectively used as an occasional second-line diagnostic supplement:

- Only where there remains clinical uncertainty as to either the current malignancy or the possible malignant potential of the pancreatic cyst based upon a comprehensive first-line evaluation; and
- A decision regarding treatment (e.g., surgery) has not already been made based on existing information.

BCR-ABL

- Breakpoint testing for BCR-ABL1 (identifies the location of a break in the BCR and ABL1 genes to help diagnose and treat leukemia) is commonly performed as a combination or panel of tests (major, minor, and other breakpoints). To report multiple tests assigned a single ID, use CPT code 81479.
- CPT code 81479 should also be used to report BCR-ABL translocation analysis by Next Generation Sequencing (NGS).

Cobas® EGFR Mutation Test

Cobas EGFR Mutation Test is reasonable and necessary for the detection of epidermal growth factor receptor (EGFR) gene for non-small cell lung cancer (NSCLC) tumor tissue. The test is intended to be used to help select patients with NSCLC for whom Tarceva® (erlotinib), an EGFR tyrosine kinase inhibitor (TKI), is indicated.

Therascreen® EGFR RGQ PCR Kit

Therascreen EGFR RGQ PCR kit is reasonable and necessary for the detection of the epidermal growth factor receptor (EGFR) gene from non-small cell lung cancer (NSCLC) tumor tissue. The test is intended to be used to select patients with NSCLC for whom GILOTRIF™ (afatinib), an EGFR tyrosine kinase inhibitor (TKI), is indicated.

Germline Testing for Use of PARP Inhibitors

The United States (U.S.) Food and Drug Administration (FDA) has approved several poly ADP-ribose polymerase (PARP) inhibitor treatments indicated for patients with ovarian cancer, breast cancer, pancreatic cancer, and prostate cancer.

Results of tests that assess for deleterious variants in homologous recombination repair (HRR) genes such as BRCA1 and 2 can be used as an aid in patients who are being considered for treatment with PARP inhibitors in accordance with published guidelines and approved therapeutic product labeling. These genes are often tested as part of routine management of these cancer patients as part of services that interrogate a panel of genes. In rare circumstances, limited testing for only a select group of genes may be tested to ensure compliance with FDA indicated drug usage, wherein additional genes outlined in guidelines such as the NCCN are not necessary because the patient does not meet testing criteria for larger panels.

Germline testing for use of PARP inhibitors is reasonable and necessary if:

- The patient meets clinical indication for immediate use of a PARP inhibitor for an FDA-approved use; and
- The patient has had no previous germline testing for hereditary cancer or somatic testing of the same cancer that included the genes necessary for testing; and
- The patient does not meet germline testing requirements per existing guidelines or standards of care requiring more comprehensive testing. (For further guidance and clinical criteria, please refer to the sourcing below for Lab-Developed Tests for Inherited Cancer Syndromes in Patients with Cancer.)

Guardant360®, Plasma-Based Genomic Profiling in Solid Tumors

Guardant360® is reasonable and necessary only when all of the following conditions are met:

- Patient has been diagnosed with a recurrent, relapsed, refractory, metastatic, or advanced solid tumor that did not originate from the central nervous system. Patients who would meet all of the indications on the FDA label for larotrectinib if they are found to have an NTRK mutation may be considered to have advanced cancer; and
- Patient has not previously been tested with the Guardant360® test for the same genetic content. For a patient who has been tested previously using Guardant360® for cancer, that patient may not be tested again unless there is clinical evidence that the cancer has evolved wherein testing would be performed for different genetic content. Specifically, in patients with previously tested cancer, who have evidence of new malignant growth despite response to a prior targeted therapy, that growth may be considered to be sufficiently genetically different to require additional genetic testing; and
- Patient is untreated for the cancer being tested, or the patient is not responding to treatment (e.g., progression or new lesions on treatment); and
- The patient has decided to seek further cancer treatment with the following conditions:
    - The patient is a candidate for further treatment with a drug that is either FDA-approved for that patient's cancer, or has an NCCN 1 or NCCN 2A recommendation for that patient's cancer; and
    - The FDA-approved indication or NCCN recommendation is based upon information about the presence or absence of a genetic biomarker tested for in the Guardant360® assay; and
- Tissue-based, CGP is infeasible (e.g., quantity not sufficient for tissue-based CGP or invasive biopsy is medically contraindicated) or specifically in NSLC Tissue-based CGP has shown no actionable mutations.

Other liquid biopsies will be reasonable and necessary for the same indications if they display similar performance in their intended used applications to Guardant360®.

InVisionFirst, Liquid Biopsy

InVisionFirst™ Lung (Inivata, Research Triangle Park, NC) (hereafter InVision) is a plasma-based, somatic comprehensive genomic profiling test (CGP) for patients with advanced (Stage IIIB/IV) non-small cell lung cancer (NSCLC) and is reasonable and necessary for the following indications:

- At diagnosis:
    - When results for EGFR single nucleotide variants (SNVs) and insertions and deletions (indels); rearrangements in ALK and ROS1; and SNVs for BRAF are not available; and
    - When tissue-based CGP is infeasible [i.e., quantity not sufficient (QNS) for tissue-based CGP or invasive biopsy is medically contraindicated]; or

- At progression:
  - For patients progressing on or after chemotherapy or immunotherapy who have not been tested for EGFR SNVs and indels; rearrangements in ALK and ROS1; and SNVs for BRAF, and for whom tissue based CGP is infeasible; or
  - For patients progressing on EGFR tyrosine kinase inhibitors (TKIs).

If no genetic alteration is detected by InVision® or if circulating tumor DNA (ctDNA) is insufficient/not detected, tissue-based genotyping should be considered.

KRAS

Two tests have met the Food and Drug Administration (FDA) criteria for KRAS genetic testing:

Therascreen® KRAS to detect 7 somatic mutations in the human KRAS oncogene was developed to aid in the identification of colorectal cancer (CRC) patients for treatment with Erbitux® (cetuximab).

Cobas® KRAS to detect mutations in codons 12 and 13 of the KRAS gene was developed to aid in identification of CRC patients for treatment with Erbitux® (cetuximab) or Vectibix® (panitumumab).

MammaPrint®

MammaPrint® is a diagnostic test that analyzes the gene expression profile of FFPE breast cancer tissue samples to assess a patients' risk for distant metastasis.

The test can be performed using either an FDA-cleared in vitro microarray assay or a next generation sequencing (NGS)-based assay.

MammaPrint® was prospectively validated as a microarray assay in the 6,693 patient MINDACT trial in early stage breast cancer, < 5cm up to 3 positive lymph nodes and independent of receptor status. The Mammaprint® NGS test has demonstrated technically equivalent performance to the predicate microarray test.

Melanoma Risk Stratification Molecular Testing

Molecular diagnostic tests used to assist in risk stratification of melanoma patients are reasonable and necessary when all of the following are true:

- The patient has a personal history of melanoma and:
  - Either:
    - Has Stage T1b and above, or
    - Has T1a with documented concern about adequacy of microstaging.
  - Is undergoing workup or being evaluated for treatment, and
  - Does not have metastatic disease, and
  - Presumed risk for a positive Sentinel Lymph Node Biopsy (SLNB) based on clinical, histological, or other information is > 5%, and
  - Has a disease stage, grade, and Breslow thickness (or other qualifying conditions) within the intended use of the test.
- The test has demonstrated, as part of a technical assessment:
  - Clinical validity of analytes tested in predicting metastatic disease (or the absence of metastatic disease) in peer-reviewed scientific literature.
  - Utility beyond clinical, histological, and radiographical factors in the ability to accurately stratify patients into risk groups to manage patient care, such by precluding unnecessary sentinel lymph node biopsies.
  - Appropriate analytical validity.
  - Performance characteristics equivalent or superior to other similar tests that are reasonable and necessary.

Note: These tests may also be reported with CPT code 81599.

Molecular Assays for the Diagnosis of Cutaneous Melanoma

The purpose of this test is to assist dermatopathologists to arrive at the correct diagnosis of melanoma versus non-melanoma when examining skin biopsies.

It is reasonable and necessary for molecular Deoxyribonucleic acid (DNA)/Ribonucleic acid (RNA) assays that aid in the diagnosis or exclusion of melanoma from a biopsy when ALL of the following clinical conditions are met:

- The test is ordered by a board-certified or board-eligible dermatopathologist.

- The specimen is a primary (non-metastatic, non-re-excision specimen) cutaneous melanocytic neoplasm for which the diagnosis is equivocal/uncertain (i.e., clear distinction between benign or malignant cannot be achieved using clinical and/or histopathological features alone) despite the performance of standard-of-care test procedures and relevant ancillary tests (i.e., immunohistochemical stains).
- The specimen includes an area representative of the lesion or portion of the lesion that is suspicious for malignancy.
- The patient may be subjected to additional intervention, such as re-excision and/or sentinel lymph node biopsy, as a result of the diagnostic uncertainty.
- The patient has not been tested with the same or similar assay for the same clinical lesion.
- The test is validated for use in the intended-use population and is performed according to its stated intended-use.
- The test demonstrates Analytical and Clinical Validity (AV and CV) and Clinical Utility (CU) and undergoes a technical assessment to demonstrate compliance of the service with this policy.
- The test demonstrates performance characteristics equivalent or superior to other similar tests that are reasonable and necessary.

Microsatellite Instability-High (MSI-H) and Mismatch Repair Deficient (dMMR) Biomarker for Patients With Unresectable or Metastatic Solid Tumors

The use of Keytruda for treatment of patients with unresectable or metastatic solid tumors having either microsatellite instability-high (MSI-H) or mismatch repair deficient (dMMR) biomarkers. Keytruda, a human PD-1 blocking antibody, is indicated for the treatment of metastatic melanoma, non-small cell lung cancer, recurrent or metastatic head and neck squamous cancer, advanced/metastatic urothelial cancer, and classical Hodgkin's lymphoma.

One of the following is allowed:
- dMMR by immunohistochemistry (IHC); or
- MSI by PCR; or
- Multi-gene NGS panel inclusive of MSI microsatellite loci, and MLH1, MSH2, MSH6 and PMS2 genes.

Testing by one of the above methodologies is reasonable and necessary if testing for dMMR or MSI has not previously been performed on the patient's tumor sample. A multi-gene NGS panel inclusive of MSI microsatellite loci and MLH1, MSH2, MSH6 and PMS2 gene is reasonable and necessary. A multi-gene NGS panel and separate MSI by PCR will be denied as not reasonable and necessary. If testing is performed by NGS, the test must be a properly designed and appropriately validated assay demonstrating 95% concordance to the reference method (MSI by PCR).
- To report a dMMR or MSI service, reference specific CPT codes.
- To report by NGS, use CPT code 81479.

ClonoSEQ® Assay

Indicated uses for ClonoSeq® include acute lymphoblastic leukemia (ALL), multiple myeloma (MM), chronic lymphocytic leukemia (CLL), diffuse large B-cell lymphoma (DLBCL), and Mantle cell lymphoma. For further guidance and coding/billing criteria, refer to the Minimal Residual Disease Testing for Cancer section below and Minimal Residual Disease Testing for Hematologic Cancers sourcing.

Minimal Residual Disease Testing for Cancer

It is reasonable and necessary for minimally invasive molecular DNA and RNA tests that detect minimal residual disease (MRD) in patients with a personal history of cancer when **all** of the following are true:
- If Next-Generation Sequencing (NGS) methodology is used in testing, the conditions set by NCD 90.2 are fulfilled (summarized: the patient has advanced cancer; plans on being treated for said cancer, and has not been previously tested with the same test for the same genetic content) or are not applicable (the patient does not have cancer as defined below). To report MRD analysis by NGS that measures multiple analytes, use CPT code 81479.
- The patient has a personal history of cancer, the type and staging of which is within the intended use of the MRD test.
- The identification of recurrence or progression of disease within the intended use population of the test is identified in the National Comprehensive Cancer Network (NCCN) or other established guidelines as a condition that requires a definitive change in patient management.
- The test is demonstrated to identify molecular recurrence or progression before there is clinical, biological or radiographical evidence of recurrence or progression and demonstrates sensitivity and specificity of subsequent recurrence or progression comparable with or superior to radiographical or other evidence (as per the standard-of-care for monitoring a given cancer type) of recurrence or progression.
- The test being utilized precludes other surveillance or monitoring tests intended to provide the same or similar information, unless they either (a) are required to follow-up or confirm the findings of this test or (b) are medically required for further assessment and management of the patient.

- If the test is to be used for monitoring a specific therapeutic response, it must demonstrate the clinical validity of its results in published literature for the explicit management or therapy indication (allowing for the use of different drugs within the same therapeutic class, so long as they are considered 'equivalent and interchangeable' for the purpose of MRD testing, as determined by national or society guidelines).
- Clinical validity (CV) of any analytes (or expression profiles) measured must be established through a study published in the peer-reviewed literature for the intended use of the test in the intended population.
- The test is being used (a) in a patient who is part of the population in which the test was analytically validated and (b) according to the intended use of the test.
- The MRD test [unless it is a FDA approved and established standard-of-care single-gene polymerase chain reaction (PCR)] satisfactorily completes a technical assessment that will evaluate and confirm that the analytical validity, clinical validity, and clinical utility criteria set in this policy are met.
- Tests utilizing a similar methodology or evaluating a similar molecular analyte to a test for which there is a generally accepted testing standard or for which existing coverage exists must demonstrate equivalent or superior test performance (i.e., sensitivity and/or specificity) when used for the same indication in the same intended-use population.

Next-Generation Sequencing for Solid Tumors and for Myeloid Malignancies and Suspected Myeloid Malignancies

Next-Generation Sequencing (NGS) is not a specific test, but a sequencing methodology utilized to capture genomic information. Unlike Sanger sequencing (the prior standard technology) that typically provides sequence information for a single deoxyribonucleic acid (DNA) strand/molecule, NGS allows for massively parallel sequencing of millions of DNA molecules concurrently. This allows for capturing many relevant genomic targets simultaneously, usually by utilizing capture technologies such as by polymerase chain reaction (PCR) amplification or hybrid capture. As such, NGS tests for use in cancer are often comprised of gene panels whose content is either relevant to a specific tumor type or condition, or a larger panel of genes that can be used for multiple tumor types, including in hematopoietic malignancies.

For Next-Generation Sequencing for solid tumors, the following must be present:

- As per NCD 90.2, this test is reasonable and necessary when the patient has:
  - Recurrent cancer, relapsed cancer, refractory cancer, metastatic cancer, or advanced cancer (stages III or IV); **and**
  - Has not been previously tested by the same test for the same genetic content; **and**
  - Is seeking further treatment.
- The test has satisfactorily completed a technical assessment for the stated indications of the test.
- The assay performed includes at least the minimum genes and genomic positions required for the identification of clinically relevant FDA-approved therapies with a companion diagnostic biomarker as well as other biomarkers known to be necessary for clinical decision making for its intended use that can be reasonably detected by the test. These genes and variants will change as the literature and drug indications evolve.

For Next-Generation Sequencing for myeloid malignancies and suspected myeloid malignancies, the following must be present:

- For tests that are specifically indicated in patients whom are known to have a myeloid malignancy at the time of testing, NCD 90.2 applies.
- The patient has a diagnosis of AML, MDS, or MPN. AML, MDS, and MPN are herein classified as refractory and/or metastatic cancers and fulfil the NCD 90.2 criteria.
- The test has satisfactorily completed a technical assessment for the stated indications of the test.
- The assay performed includes at least the minimum genes and positions indicated for its intended use.
- For patients that do not have a diagnosis of a myeloid malignancy, where one is suspected, the patient must have an undefined cytopenia for greater than 4 months, other possible causes have been reasonably excluded.
- Testing is performed on bone marrow biopsies, bone marrow aspirates, bone marrow clots, peripheral blood samples, or extramedullary sites suspected of harboring a myeloid malignancy.

Targeted and Comprehensive Genomic Profile Next Generation Sequencing (NGS) Testing

Targeted Next-Generation Sequencing (NGS) panels are defined as tests that identify somatic alterations known to occur in certain regions (i.e., 'hotspots') within specific genes of interest for cancer management (i.e., diagnosis, selection of molecularly targeted therapies, prognosis in a context where prognostic classification is essential for treatment selection). Generally, these NGS panels can detect single nucleotide variants (SNVs) and small insertions or deletions (INDELs) within these regions. These alterations typically represent response or lack of response to corresponding targeted cancer

therapies. The hotspot test should include relevant regions in the genes required for companion diagnostic testing and/or known to be necessary for proper patient management.

Comprehensive Genomic Profile (CGP) refers to NGS-based molecular assays that provide additional insight beyond individual gene hotspots; these assays seek to describe the genomic makeup of a tumor and can help identify underlying mechanisms of disease to guide clinical decision making. These tests include not only mutations in individual relevant genes, but also patterns of mutations across related genes in established cancer pathways and often include an assessment of overall mutational burden. These tests typically involve sequencing of entire exonic regions of genes of interest (within a comprehensive gene panel or whole exome sequencing) and may also include selected intronic regions. CGP can detect multiple types of molecular alterations (i.e., SNVs, small and large INDELs, copy number alterations (CNAs), structural variants (SVs), and splice-site variants) in a single assay. Patterns of mutations seen across multiple genes may be used to infer clinically relevant etiologies, such as DNA mismatch repair deficiency and microsatellite instability, and total mutational load/burden (TMB) may be determined. CGP testing may also include RNA sequencing to detect structural variations, such as translocations or large deletions, and to detect functional splicing mutations. CGP is not defined as a targeted panel.

Laboratories with 2 to 4 gene(s) on their targeted NGS panel should use CPT 81479. For other targeted NGS gene panel services for somatic variant detection, more specific CPT codes exist.

CGP testing is not defined as a targeted panel, and it is a test not currently described by any existing CPT code. Therefore, to report a CGP service, use CPT code 81479. Coverage of CGP is limited to one test per surgical specimen and precludes the use of any other molecular testing on that specimen.

For NGS-based tests that do not fit under the above definitions of “targeted” or “Comprehensive” panels, reporting CPT code 81479 is appropriate.

Molecular Biomarkers for Risk Stratification of Indeterminate Pulmonary Nodules Following Bronchoscopy

Molecular tests are reasonable and necessary to aid in the diagnosis or exclusion of lung cancer in a patient with an indeterminate pulmonary nodule (IPN) following a non-diagnostic bronchoscopy when ALL of the following conditions are met:

- The patient has undergone bronchoscopy for an indeterminate pulmonary nodule, **and**
  - The bronchoscopy has failed to provide a specific histopathological diagnosis such that further diagnostic procedures would otherwise be necessary to pursue a specific diagnosis (non-diagnostic bronchoscopy); **and**
  - Test results will be used to meaningfully inform patient management within the framework of nationally recognized consensus guidelines.
  - The nodule cannot or will not be evaluated by an alternate methodology (EBUS, FNA, etc.) for a specific diagnosis prior to receipt of molecular test results.
- The patient does NOT have any of the following:
  - Personal history of lung cancer.
  - Current diagnosis of cancer or high clinical suspicion for cancer.
  - An overall low risk for pulmonary malignancy such that test results would not meaningfully alter patient management and significantly improve patient outcomes.
  - An overall high risk for pulmonary malignancy such that test results would not meaningfully alter patient management and significantly improve patient outcomes.
- The patient has not been tested with the same or similar assay for the same clinical indication.
- The patient is within the population and has the indication for which the test was developed and is reasonable and necessary. The lab providing the test is responsible for clearly indicating to treating clinicians the population and indication for test use.
- The test has demonstrated clinical validity and utility, establishing a clear and significant biological/molecular basis for stratifying patients and subsequently selecting (either positively or negatively) a clinical management decision in a clearly defined population.
- Clinical validity of any analytes (or expression profiles) measured must be established through a study published in the peer-reviewed literature for the intended use of the test in the intended population.
- Rule-out tests should have a high sensitivity and negative predictive value (NPV) such that patients can be safely selected for a less aggressive management strategy without delay to diagnosis due to false negative results.
- Rule-in tests should have a high specificity and positive predictive value (PPV) such that patients can be safely selected for more aggressive management without significantly increasing procedures in patients without cancer due to false positive results.

- The test demonstrates analytical validity including both analytical and clinical validations. If the test relies on an algorithm (which may range in complexity from a threshold determination of a single numeric value to a complex mathematical or computational function), the algorithm must be validated in a cohort that is not a development cohort for the algorithm.
- Tests utilizing a similar methodology or evaluating a similar molecular analyte to a test for which there is a generally accepted testing standard or for which existing coverage exists must demonstrate equivalent or superior test performance (i.e., sensitivity and/or specificity) when used for the same indication in the same intended-use population. New tests that become available with significantly improved performance may render older tests no longer compliant with this policy.
- The test successfully completes a technical assessment that ensures the test is reasonable and necessary as described above.

Pharmacogenomics Testing (PGx)

PGx tests are indicated when medications are being considered for use (or already being administered) that are reasonable and necessary, appropriate, and approved for use in the patient's condition and are known to have a gene(s) drug interaction that has been demonstrated to be clinically actionable as defined by the FDA (PGx information required for safe drug administration) or Clinical Pharmacogenetic Implementation Consortium (CPIC) guidelines (Category A and B).

The selection of the medications in question must be derived from clinical factors/necessity rather than from a PGx test. Once the putative therapeutic agents are selected, and those agents are known to have gene-drug interactions as identified above, then a PGx test is reasonable and necessary when the result of that test is necessary for the physician's decision-making process regarding safely administering or dosing the drug.

PGx testing is not reasonable and necessary merely on the basis of a patient having a particular diagnosis. Unless the record reflects that the treating clinician has already considered non-genetic factors to make a preliminary drug selection, PGx testing is not reasonable and necessary.

PGx testing is not reasonable and necessary when a treating clinician is not considering treatment with a medication that has an actionable drug-gene interaction, or when the use of a medication with a drug-gene interaction is not reasonable and necessary.

If a treating clinician orders a single gene test or a test for a particular allele(s), but as a matter of operational practicality, the laboratory tests that single gene or allele on a platform that looks for variants in other genes / alleles as well, that particular test done in that particular instance is considered a single gene / allele test for coverage purposes. In this scenario the provider may bill for the component of the test that was reasonable and necessary (in this example, the single gene test).

A multi-gene panel is reasonable and necessary if more than one single gene on that panel would be reasonable and necessary for safe use of the medication in question or if multiple drugs are being considered (each fulfilling the criteria of actionable gene-drug interactions) that have different relevant genes. Additionally, a gene panel must contain at a minimum all the necessary relevant gene/allele content required for their indicated use to meet clinical utility requirements. Such minimum criteria are determined by experts including relevant associations such as the Association for Molecular Pathology and are considered during the technical assessment. A multi-gene panel is not reasonable and necessary if only a single gene on the panel is reasonable and necessary.

The patient has a diagnosis for which pharmacologic therapy is reasonable and necessary, and the drug or drugs that the clinician is considering using must be reasonable and necessary for the treatment of the patient's diagnosis.

The clinician has made an initial personalized decision for the patient based on the patient's diagnosis, the patient's other medical conditions, other medications the patient is taking, professional judgement, clinical science and basic science pertinent to the drug (e.g., mechanism of action, side effects), the patient's past medical history and when pertinent family history and the patient's preferences and values.

The provider performing the service must have a record of what drug(s) is/are being considered and for what indication(s) to ensure the test performed is reasonable and necessary.

Genes not identified as having actionable use are not reasonable and necessary. The algorithms employed in combinatorial testing are also not currently reasonable and necessary components of multi-gene testing.

If no CPT code is available for the gene being tested, the code 81479 may be used.

The following do not have a specific CPT code available:
- BCHE
- CACNA1S
- CYP2B6
- CYP4F2
- IFNL4
- NAT
- NAT2
- NeuroIDgenetix

For further guidance and clinical criteria, please refer to the Pharmacogenomics Testing sourcing below.

Phenotypic Biomarker Detection From Circulating Tumor Cells

Assays that detect biomarkers from circulating tumor cells (CTCs) are reasonable and necessary when all of the following are met:
- The patient has been diagnosed with cancer.
- The specific cancer type has an associated biomarker.
- The associated biomarker has already established clinical utility (CU) in the peer-reviewed published literature for the intended cancer type and for the specific indication in the intended patient population.
  - The biomarker's CU may include any of the following: it can be used to diagnose, risk-stratify, predict, or monitor response to therapy, as recommended by national or society guidelines (i.e., American Society of Clinical Oncology (ASCO), National Comprehensive Cancer Network (NCCN)).
- At least 1 of the following criteria are met:
  - The patient's cancer has not previously been tested for the specific biomarker; **or**
  - The patient has newly metastatic cancer, and a metastatic lesion has not been tested for the specific biomarker; **or**
  - The patient demonstrates signs of clinical, radiological, or pathologic disease progression; **or**
  - There is concern for resistance to treatment based on specific and well-established clinical indications.
- Testing for the biomarker can be performed using CTCs.
- The CTC-based biomarker test successfully completes a comprehensive technical assessment that will ensure that Analytical Validity (AV) (including an analytical and clinical validation), Clinical Validity (CV), and CU criteria are met to establish the test as reasonable and necessary.
  - The clinical validation has demonstrated performance that is equivalent or superior to tissue-based testing or another already-accepted test for the same biomarker for the same intended use.
  - CV (for new analytes) must be established through studies published in the peer-reviewed literature for the intended use of the test in the intended population.
- Tissue-based testing for the specific biomarker is infeasible (e.g., quantity not sufficient or invasive biopsy is medically contraindicated) or will not provide sufficient information for subsequent medical management (e.g., in cases where human epidermal growth factor receptor 2 (HER2) overexpression is negative in a tissue biopsy but may be positive in the CTCs, due to tumor heterogeneity).
- Duplicate testing of the same biomarker (from the same sample type and for the same clinical indication) using different methodologies is not reasonable and necessary. For example, testing for androgen receptor splice variant 7 (AR-V7) from CTCs by messenger RNA (mRNA) as well as immunohistochemistry (IHC)-based methodologies, for the same clinical indication, is not reasonable and necessary.

The following tests are reasonable and necessary:
- Biocept Target Selector HER2 Assay.
- Androgen Receptor Variant (AR-V7) Protein Test.

Predictive Classifiers for Early-Stage Non-Small Cell Lung Cancer (NSCLC), Razor 14-Gene Lung Cancer Assay

The use of molecular diagnostic laboratory tests as a predictive classifier for NSCLC are reasonable and necessary when all of the following criteria are met:
- The patient has a non-squamous NSCLC with a tumor size < 5cm, and there are no positive lymph nodes (i.e., American Joint Committee on Cancer Eighth Edition Stages I and IIa).
- The patient is sufficiently healthy to tolerate chemotherapy.

- Adjuvant platinum-containing chemotherapy is being considered for the patient.
- The test is ordered by a physician who is treating the patient for NSCLC (generally a medical oncologist, surgeon, or radiation oncologist) to help in the decision of whether or not to recommend adjuvant chemotherapy.

ProMark Risk Score

ProMark (Metamark Genetics) is reasonable and necessary to help determine which patients with early stage needle biopsy proven prostate cancer can be conservatively managed rather than treated with definitive surgery or radiation therapy.

Prognostic and Predictive Molecular Classifiers for Bladder Cancer

Molecular diagnostic tests for use in a patient with bladder cancer are reasonable and necessary when all of the following conditions are met:

- The patient is being actively managed for bladder cancer.
- The patient is within the population and has the indication for which the test was developed and is reasonable and necessary. The laboratory will make available the appropriate indications of the test to the treating/ordering physician.
- At least 1 of the 2 criteria are met:
  - The patient is a candidate for multiple potential treatments, which could be considered to have varied or increasing levels of intensity based on a consensus guideline, and the physician and patient must decide among these treatments; **or**
  - The patient is a candidate for multiple therapies, and the test has shown that it predicts response to a specific therapy among accepted therapy options based on nationally recognized consensus guidelines (i.e., National Comprehensive Cancer Network [NCCN], American Society of Clinical Oncology [ASCO], Society of Urologic Oncology [SUO], or American Urological Association [AUA]).
- If Next-Generation Sequencing (NGS) methodology is used in testing, the conditions set by NCD 90.2 are fulfilled (summarized: the patient has advanced cancer; plans on being treated for said cancer and has not been previously tested with the same test for the same genetic content).
- The test demonstrates analytical validity including both analytical and clinical validations. If the test relies on an algorithm (which may range in complexity from a threshold determination of a single numeric value to a complex mathematical or computational function), the algorithm must be validated in a cohort that is not a development cohort for the algorithm.
- The test has demonstrated clinical validity and utility, establishing a clear and significant biological/molecular basis for stratifying patients and subsequently selecting (either positively or negatively) a clinical management decision (in the bullet above) in a clearly defined population.
- The test successfully completes a technical assessment that ensures the test is reasonable and necessary as described above.
- The genomic content interrogated by the test must be relevant to the therapy under consideration.

Solid Organ Allograft Rejection

It is reasonable and necessary for molecular diagnostic tests used in the evaluation and management of patients who have undergone solid organ transplantation. These tests can inform decision making along with standard clinical assessments in their evaluation of organ injury for active rejection (AR).

These tests may be ordered by qualified physicians considering the diagnosis of AR affiliated with a transplant center, helping to rule in or out this condition when assessing the need for or results of a diagnostic biopsy. They should be considered along with other clinical evaluations and results and may be particularly useful in patients with significant contraindications to invasive procedures.

Molecular diagnostic tests that assess a transplanted allograft for rejection status are reasonable and necessary when all of the following criteria are met:

- The test must provide information about at least one of the two following clinical status determinations:
  - AR status.
  - Cellular or Antibody-mediated rejection (ACR or AMR) status.
- The test demonstrates analytical validity (AV), including an analytical and clinical validation for any given measured analytes, and has demonstrated equivalence or superiority for sensitivity or specificity (depending on intended use) of detecting allograft rejection to other already-accepted tests for the same intended use measuring the same or directly comparable analytes.
- Clinical validity (CV) of any analytes (or expression profiles) measured must be established through a study published in the peer-reviewed literature for the intended use of the test in the intended population. The degree of validity must

be similar or superior to established and tests that are reasonable and necessary (refer to associated coverage articles).

Molecular Testing for Risk Stratification of Thyroid Nodules

Molecular diagnostic tests are reasonable and necessary for use in a patient with an indeterminate or suspicious thyroid nodule when all the following criteria are met:

- The patient:
  - Has an index nodule that has not been tested with the same or similar assay for the same clinical indication, and
    - The nodule is indeterminate as defined by Bethesda categories III - IV; or
    - The nodule is Bethesda category V and molecular testing may aid in further stratifying the type of malignancy.
- If the patient has multiple nodules, concurrent or reflex testing may be reasonable and necessary, provided the above criteria are also met.
- The results of the test will be used to aid in surgical decision making after a consideration of clinical, radiographic and cytologic features.
- The patient is within the population and has the indication for which the test was developed. The laboratory providing the test is responsible for clearly indicating to treating clinicians the population and indication for test use.
- The test demonstrates analytical validity, including both analytical and clinical validation, on a cohort of patients appropriate for its intended use. If the test relies on an algorithm, the algorithm must be validated in a cohort that is not a development cohort for the algorithm.
- The test has demonstrated clinical validity and utility in peer-reviewed, published literature, establishing a clear and significant biological/molecular basis for stratifying patients and subsequently selecting (either positively or negatively) a clinical management decision in a clearly defined population.
- The test successfully completes a technical assessment that ensures the test is reasonable and necessary as described above.
- The performance characteristics of the test have been demonstrated to be as good or better than services that are currently reasonable and necessary.

Gene Expression Profile Tests for Decision-Making in Castration Resistant and Metastatic Prostate Cancers

Gene expression profile tests that assess risk or predict therapeutic response in men who have an established diagnosis of castration resistant or metastatic prostate cancer is reasonable and necessary to help guide treatment decisions in men with prostate cancer and a life expectancy such that they are candidates for prostate cancer treatment according to the most recent nationally recognized guidelines at the time of testing or based on FDA labelling of drugs and biologics available as potential treatment options.

Reasonable and necessary criteria include:

- Both of the following criteria must be met:
  - The patient is being actively managed for castration resistant or metastatic (hormone sensitive or castration resistant) prostate cancer.
  - The patient is within the population and has the indication for which the test was developed and validated.
- At least 1 of the following criteria are met:
  - The patient is a candidate for more than one management option, which could be considered to have varied, or increasing/decreasing levels of intensity based on a nationally recognized consensus guideline, and the physician and patient must decide among these treatments, or
  - The patient is a candidate for more than one management option, and the test has shown that it predicts response to a specific therapy among accepted therapy options based on nationally recognized consensus guidelines and/or FDA labelling.
- The patient has not been tested with the same or similar test for the same intended use.
- The patient has not received pelvic radiation or androgen deprivation therapy (ADT) prior to the biopsy or prostate resection specimen on which the test will be performed. The only exception to this is for men who are naïve to secondary systemic therapies (that could be given after ADT monotherapy) AND at least 1 of the following is true:
  - They do not have other standard-of-care drug-targetable gene alterations to guide systemic therapy, as defined in nationally recognized guidelines, or
  - They have other standard-of-care drug-targetable gene alterations to guide systemic therapy, as defined in nationally recognized guidelines but they are not eligible for those therapies for another reason.
- The test demonstrates analytical validity (AV), clinical validity (CV) and clinical utility (CU), establishing a clear and significant biological/ molecular basis for stratifying patients and subsequently selecting (either positively or negatively) a clinical management in a clearly defined population.

- Clinical validity of any analytes (or expression profiles) measured must be established through a study published in the peer-reviewed literature for the intended use of the test in the intended population.
- If the test relies on an algorithm, the algorithm must be validated in a cohort that is not a development cohort for the algorithm.
- The test successfully completes a technical assessment that ensures that AV, CV, and CU criteria set in this policy are met to establish the test as reasonable and necessary.

Genomic expression profile tests that demonstrate equivalent or superior analytical and clinical validity to other similar tests will be reasonable and necessary for the same indications.

Covered Indications for CPT Code 81599

Solid Organ Allograft Rejection

Refer to criteria above under CPT code 81479.

Melanoma Risk Stratification Molecular Testing

Refer to criteria above under CPT code 81479.

Non-Covered Indications for CPT Code 81479

The test descriptions are appropriate to be reported with the unlisted code; however, the following tests are unlikely to impact therapeutic decision-making or directly impact treatment, outcome, and/or clinical management in the care of the Medicare member and will be denied as not reasonable and necessary:

- BluePrint® Test.
- Molecular Biomarker Testing for Risk Stratification of Cutaneous Squamous Cell Carcinoma.
- HAX1 gene sequencing and panels of tests that include the HAX1 gene.
- Serotonin Transporter genotyping (HTTLPR)/ HTTLPR Gene Testing.
- KIF6 genotype test.
- LPA-Intron 25 genotype test.
- Prometheus IBD sgi Diagnostic Test.
- SULT4A1 Genetic Testing and panels of tests that include the SULT4A1 gene.
- VEGFR2 testing and panels of tests that include the VEGFR2 receptor.

Nationally Non-Covered Indications

Compliance with the provisions in this policy is subject to monitoring by post payment data analysis and subsequent medical review. Title XVIII of the Social Security Act, Section 1862(a)(1)(A) states " ...no Medicare payment shall be made for items or services which are not reasonable and necessary for the diagnosis and treatment of illness or injury...". Furthermore, it has been longstanding CMS policy that **"tests that are performed in the absence of signs, symptoms, complaints, or personal history of disease or injury are not covered unless explicitly authorized by statute".**

Screening services such as pre - symptomatic genetic tests and services used to detect an undiagnosed disease or disease predisposition are not a Medicare benefit and not covered. Similarly, Medicare may not reimburse the costs of tests/examinations that assess the risk of a condition unless the risk assessment clearly and directly effects the management of the patient.

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; however, language may be included in the listing below to indicate if a code is non-covered. Benefit coverage for health services is determined by the member specific benefit plan document and applicable laws that may require coverage for a specific service. The inclusion of a code does not imply any right to reimbursement or guarantee claim payment. Other Policies and Guidelines may apply.

CPT Code	Description
81479	Unlisted molecular pathology procedure
81599	Unlisted multianalyte assay with algorithmic analysis

CPT Code	Description
84999	Unlisted chemistry procedure

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Diagnosis Code	Description
Molecular Pathology/Genetic Testing Reported with Unlisted Codes: Diagnosis Codes	

Non-Covered Diagnosis Code

Non-Covered Diagnosis Codes List

This list contains diagnosis codes that are **never covered when given as the primary reason for the test.** If a code from this section is given as the reason for the test and you know or have reason to believe the service may not be covered, call UnitedHealthcare to issue an Integrated Denial Notice (IDN) to the member and you. The IDN informs the member of their liability for the non-covered service or item and appeal rights. You must make sure the member has received the IDN prior to rendering or referring for non-covered services or items in order to collect payment.

Centers for Medicare and Medicaid Services (CMS) Related Documents

After checking the table below and searching the Medicare Coverage Database, if no NCD, LCD, or LCA is found, refer to the criteria as noted in the Coverage Rationale section above.

NCD	LCD	LCA	Contractor Type	Contractor Name
N/A	L35396 Biomarkers for Oncology	A52986 Billing and Coding: Biomarkers for Oncology	Part A and B MAC	Novitas**
N/A	L39017 MolDX: Lab-Developed Tests for Inherited Cancer Syndromes in Patients with Cancer	A54689 Billing and Coding: Germline testing for use of PARP inhibitors	Part A and B MAC	CGS
N/A	L38966 MolDX: Lab-Developed Tests for Inherited Cancer Syndromes in Patients with Cancer	A54338 Billing and Coding: MolDX: Germline testing for use of PARP inhibitors	Part A and B MAC	Palmetto**
N/A	L35160 MolDX: Molecular Diagnostic Tests (MDT)	A55294 Billing and Coding: MolDX: Germline testing for use of PARP inhibitors	Part A and B MAC	Noridian
N/A	L36256 MolDX: Molecular Diagnostic Tests (MDT)	A55295 Billing and Coding: MolDX: Germline testing for use of PARP inhibitors	Part A and B MAC	Noridian
N/A	L39040 MolDx: Lab-Developed Tests for Inherited Cancer Syndromes in Patients with Cancer	A55224 Billing and Coding: MolDX: Germline testing for use of PARP inhibitors	Part A and B MAC	WPS*
N/A	N/A	A58192 Billing and Coding: Guardant360®	Part A and B MAC	Noridian
N/A	N/A	A58214 Billing and Coding: Guardant360®	Part A and B MAC	Noridian
N/A	L38065 MolDX: Plasma-Based Genomic Profiling in Solid Tumors	A57917 Billing and Coding: MolDX: Plasma-Based Genomic Profiling in Solid Tumors	Part A and B MAC	CGS

NCD	LCD	LCA	Contractor Type	Contractor Name
N/A	L39230 MolDX: Plasma-Based Genomic Profiling in Solid Tumors	A58973 Billing and Coding: MolDX: Plasma-Based Genomic Profiling in Solid Tumors	Part A and B MAC	Noridian
N/A	L39232 MolDX: Plasma-Based Genomic Profiling in Solid Tumors	A58975 Billing and Coding: MolDX: Plasma-Based Genomic Profiling in Solid Tumors	Part A and B MAC	Noridian
N/A	L38043 MolDX: Plasma-Based Genomic Profiling in Solid Tumors	A57867 Billing and Coding: MolDX: Plasma-Based Genomic Profiling in Solid Tumors	Part A and B MAC	Palmetto**
N/A	L38168 MolDX: Plasma-Based Genomic Profiling in Solid Tumors	A57936 Billing and Coding: MolDX: Plasma-Based Genomic Profiling in Solid Tumors	Part A and B MAC	WPS*
N/A	L34864 Loss-of-Heterozygosity Based Topographic Genotyping with PathfinderTG ®	A56897 Billing and Coding: Loss-of-Heterozygosity Based Topographic Genotyping with Pathfinder TG®	Part A and B MAC	Novitas**
N/A	L36815 MolDX: Genetic Testing for BCR-ABL Negative Myeloproliferative Disease	A55233 Billing and Coding: MolDx: BCR-ABL A57570 Billing and Coding: MolDX: Genetic Testing for BCR-ABL Negative Myeloproliferative Disease	Part A and B MAC	WPS*
N/A	L36044 MolDX: Genetic Testing for BCR-ABL Negative Myeloproliferative Disease	A53531 Billing and Coding: MolDX: BCR-ABL A56959 Billing and Coding: MolDX: Genetic Testing for BCR-ABL Negative Myeloproliferative Disease	Part A and B MAC	Palmetto**
N/A	L36180 MolDX: Genetic Testing for BCR-ABL Negative Myeloproliferative Disease	A55595 Billing and Coding: MolDX: BCR-ABL A57421 Billing and Coding: MolDX: Genetic Testing for BCR-ABL Negative Myeloproliferative Disease	Part A and B MAC	Noridian
N/A	L36186 MolDX: Genetic Testing for BCR-ABL Negative Myeloproliferative Disease	A55600 Billing and Coding: MolDX: BCR-ABL A57422 Billing and Coding: MolDX: Genetic Testing for BCR-ABL Negative	Part A and B MAC	Noridian
N/A	L36117 MolDX: Genetic Testing for BCR-ABL	A54686 Billing and Coding: MolDX: BCR-ABL	Part A and B MAC	CGS

NCD	LCD	LCA	Contractor Type	Contractor Name
	Negative Myeloproliferative Disease	A56999 Billing and Coding: MolDX: Genetic Testing for BCR-ABL Negative Myeloproliferative Disease		
N/A	L36807 MolDX: Molecular Diagnostic Tests (MDT)	A55146 Billing and Coding: MolDX: BluePrint® Test	Part A and B MAC	WPS*
N/A	L35025 MolDX: Molecular Diagnostic Tests (MDT)	A53484 Billing and Coding: MolDX: BluePrint® Test	Part A and B MAC	Palmetto**
N/A	N/A	A55115 Billing and Coding: MolDX: BluePrint® Test	Part A and B MAC	Noridian
N/A	N/A	A55116 Billing and Coding: MolDX: BluePrint® Test	Part A and B MAC	Noridian
N/A	L36021 MolDX: Molecular Diagnostic Tests (MDT)	A54257 Billing and Coding: MolDX: BluePrint® Test	Part A and B MAC	CGS
N/A	L36021 MolDX: Molecular Diagnostic Tests (MDT)	A54189 Billing and Coding: MolDX: cobas® EGFR Mutation Test Guidelines	Part A and B MAC	CGS
N/A	L36021 MolDX: Molecular Diagnostic Tests (MDT)	A54199 Billing and Coding: MolDX: Therascreen® EGFR RGQ PCR Kit Guidelines	Part A and B MAC	CGS
N/A	L36807 MolDX: Molecular Diagnostic Tests (MDT)	A55162 Billing and Coding: MolDX: FDA-Approved KRAS Tests	Part A and B MAC	WPS*
N/A	L35025 MolDX: Molecular Diagnostic Tests (MDT)	A54472 Billing and Coding: MolDX: FDA-Approved KRAS Tests	Part A and B MAC	Palmetto**
N/A	L35160 MolDX: Molecular Diagnostic Tests (MDT)	A54498 Billing and Coding: MolDX: FDA-Approved KRAS Tests	Part A and B MAC	Noridian
N/A	L36256 MolDX: Molecular Diagnostic Tests (MDT)	A54500 Billing and Coding: MolDX: FDA-Approved KRAS Tests	Part A and B MAC	Noridian
N/A	L36021 MolDX: Molecular Diagnostic Tests (MDT)	A54688 Billing and Coding: MolDX: FDA-Approved KRAS Tests	Part A and B MAC	CGS
N/A	L39648 MolDX: Gene Expression Profile Tests for Decision-Making in Castration Resistant and Metastatic Prostate Cancers **Effective 09/22/2024**	A59472 Billing and Coding: MolDX: Gene Expression Profile Tests for Decision-Making in Castration Resistant and Metastatic Prostate Cancers **Effective 09/22/2024**	Part A and B MAC	CGS

NCD	LCD	LCA	Contractor Type	Contractor Name
N/A	L39686 MolDX: Gene Expression Profile Tests for Decision-Making in Castration Resistant and Metastatic Prostate Cancers **Effective 09/22/2024**	A59513 Billing and Coding: MolDX: Gene Expression Profile Tests for Decision-Making in Castration Resistant and Metastatic Prostate Cancers **Effective 09/22/2024**	Part A and B MAC	Noridian
N/A	L39688 MolDX: Gene Expression Profile Tests for Decision-Making in Castration Resistant and Metastatic Prostate Cancers **Effective 09/22/2024**	A59515 Billing and Coding: MolDX: Gene Expression Profile Tests for Decision-Making in Castration Resistant and Metastatic Prostate Cancers **Effective 09/22/2024**	Part A and B MAC	Noridian
N/A	L39636 MolDX: Gene Expression Profile Tests for Decision-Making in Castration Resistant and Metastatic Prostate Cancers **Effective 09/22/2024**	A59462 Billing and Coding: MolDX: Gene Expression Profile Tests for Decision-Making in Castration Resistant and Metastatic Prostate Cancers **Effective 09/22/2024**	Part A and B MAC	Palmetto**
N/A	L39709 MolDX: Gene Expression Profile Tests for Decision-Making in Castration Resistant and Metastatic Prostate Cancers **Effective 09/22/2024**	A59549 Billing and Coding: MolDX: Gene Expression Profile Tests for Decision-Making in Castration Resistant and Metastatic Prostate Cancers **Effective 09/22/2024**	Part A and B MAC	WPS*
N/A	L36021 MolDX: Molecular Diagnostic Tests (MDT)	A54269 Billing and Coding: MolDX: HTTLPR Gene Testing	Part A and B MAC	CGS
N/A	N/A	A55264 Billing and Coding: MolDX: HTTLPR Gene Testing	Part A and B MAC	Noridian
N/A	N/A	A55265 Billing and Coding: MolDX: HTTLPR Gene Testing	Part A and B MAC	Noridian
N/A	L35025 MolDX: Molecular Diagnostic Tests (MDT)	A53480 Billing and Coding: MolDX: HTTLPR Gene Testing	Part A and B MAC	Palmetto**
N/A	L36807 MolDX: Molecular Diagnostic Tests (MDT)	A55169 Billing and Coding: MolDX: HTTLPR Gene Testing	Part A and B MAC	WPS*
N/A	L37903 MolDX: Inivata, InVisionFirst, Liquid Biopsy for Patients with Lung Cancer	A56982 Billing and Coding: MolDX: Inivata™, InVisionFirst®, Liquid Biopsy for Patients with Lung Cancer	Part A and B MAC	CGS

NCD	LCD	LCA	Contractor Type	Contractor Name
N/A	L37897 MolDX: Inivata™, InVisionFirst®, Liquid Biopsy for Patients with Lung Cancer	A57664 Billing and Coding: MolDX: Inivata™, InVisionFirst®, Liquid Biopsy for Patients with Lung Cancer	Part A and B MAC	Noridian
N/A	L37899 MolDX: Inivata™, InvisionFirst®, Liquid Biopsy for Patients with Lung Cancer	A57665 Billing and Coding: MolDX: Inivata™, InVisionFirst®, Liquid Biopsy for Patients with Lung Cancer	Part A and B MAC	Noridian
N/A	L37870 MolDX: Inivata™, InVisionFirst®, Liquid Biopsy for Patients with Lung Cancer	A56924 Billing and Coding: MolDX: Inivata™, InVisionFirst®, Liquid Biopsy for Patients with Lung Cancer	Part A and B MAC	Palmetto**
N/A	L37921 MolDX: Inivata™, InVisionFirst®, Liquid Biopsy for Patients with Lung Cancer	A56333 Billing and Coding: Inivata™, InvisionFirst®, Liquid Biopsy for Patients with Lung Cancer	Part A and B MAC	WPS*
N/A	L36021 MolDX: Molecular Diagnostic Tests (MDT)	A54272 Billing and Coding: MolDX: KIF6 Genotype	Part A and B MAC	CGS
N/A	L36021 MolDX: Molecular Diagnostic Tests (MDT)	A54194 Billing and Coding: MolDX: MammaPrint	Part A and B MAC	CGS
N/A	L35160 MolDX: Molecular Diagnostic Tests (MDT)	A54445 Billing and Coding: MolDX: MammaPrint	Part A and B MAC	Noridian
N/A	L36256 MolDX: Molecular Diagnostic Tests (MDT)	A54447 Billing and Coding: MolDX: MammaPrint	Part A and B MAC	Noridian
N/A	L35025 MolDX: Molecular Diagnostic Tests (MDT)	A53104 Billing and Coding: MolDX: MammaPrint	Part A and B MAC	Palmetto**
N/A	L36807 MolDX: Molecular Diagnostic Tests (MDT)	A55175 Billing and Coding: MolDX: MammaPrint®	Part A and B MAC	WPS*
N/A	L36021 MolDX: Molecular Diagnostic Tests (MDT)	A54278 Billing and Coding: MolDX: MECP2 Genetic Testing	Part A and B MAC	CGS
N/A	L38016 MolDX: Melanoma Risk Stratification Molecular Testing	A57165 Billing and Coding: MolDX: Melanoma Risk Stratification Molecular Testing	Part A and B MAC	CGS
N/A	L37750 MolDX: Melanoma Risk Stratification Molecular Testing	A57268 Billing and Coding: MolDX: Melanoma Risk Stratification Molecular Testing	Part A and B MAC	Noridian

NCD	LCD	LCA	Contractor Type	Contractor Name
N/A	L37748 MolDX: Melanoma Risk Stratification Molecular Testing	A57290 Billing and Coding: MolDX: Melanoma Risk Stratification Molecular Testing	Part A and B MAC	Noridian
N/A	L37725 MolDX: Melanoma Risk Stratification Molecular Testing	A56961 Billing and Coding: MolDX: Melanoma Risk Stratification Molecular Testing	Part A and B MAC	Palmetto**
N/A	L38018 MolDX: Melanoma Risk Stratification Molecular Testing	A56636 Billing and Coding: MolDX: Melanoma Risk Stratification Molecular Testing	Part A and B MAC	WPS*
N/A	L36021 MolDX: Molecular Diagnostic Tests (MDT)	A56106 Billing and Coding: MolDX: Microsatellite Instability-High (MSI-H) and Mismatch Repair Deficient (dMMR) Biomarker for Patients with Unresectable or Metastatic Solid Tumors	Part A and B MAC	CGS
N/A	L35160 MolDX: Molecular Diagnostic Tests (MDT)	A56103 Billing and Coding: MolDX: Microsatellite Instability-High (MSI-H) and Mismatch Repair Deficient (dMMR) Biomarker for Patients with Unresectable or Metastatic Solid Tumors	Part A and B MAC	Noridian
N/A	L36256 MolDX: Molecular Diagnostic Tests (MDT)	A56104 Billing and Coding: MolDX: Microsatellite Instability-High (MSI-H) and Mismatch Repair Deficient (dMMR) Biomarker Billing and Coding Guidelines for Patients with Unresectable or Metastatic Solid Tumors	Part A and B MAC	Noridian
N/A	L35025 MolDX: Molecular Diagnostic Tests (MDT)	A56072 Billing and Coding: MolDX: Microsatellite Instability-High (MSI-H) and Mismatch Repair Deficient (dMMR) Biomarker for Patients with Unresectable or Metastatic Solid Tumors	Part A and B MAC	Palmetto**
NCD 90.2 Next Generation Sequencing (NGS)	L38822 MolDX: Minimal Residual Disease Testing for Cancer	A58434 Billing and Coding: MolDX: Minimal Residual Disease Testing for Solid Tumor Cancers	Part A and B MAC	CGS

NCD	LCD	LCA	Contractor Type	Contractor Name
		A58998 Billing and Coding: MolDX: Minimal Residual Disease Testing for Hematologic Cancers		
NCD 90.2 Next Generation Sequencing (NGS)	L38814 MolDX: Minimal Residual Disease Testing for Cancer	A58454 Billing and Coding: MolDX: Minimal Residual Disease Testing for Solid Tumor Cancers	Part A and B MAC	Noridian
		A58996 Billing and Coding: MolDX: Minimal Residual Disease Testing for Hematologic Cancers		
NCD 90.2 Next Generation Sequencing (NGS)	L38816 MolDX: Minimal Residual Disease Testing for Cancer	A58456 Billing and Coding: MolDX: Minimal Residual Disease Testing for Solid Tumor Cancers	Part A and B MAC	Noridian
		A58997 Billing and Coding: MolDX: Minimal Residual Disease Testing for Hematologic Cancers		
NCD 90.2 Next Generation Sequencing (NGS)	L38779 MolDX: Minimal Residual Disease Testing for Cancer	A58376 Billing and Coding: MolDX: Minimal Residual Disease Testing for Solid Tumor Cancers	Part A and B MAC	Palmetto**
		A58988 Billing and Coding: MolDX: Minimal Residual Disease Testing for Hematologic Cancers		
NCD 90.2 Next Generation Sequencing (NGS)	L38835 MolDX: Minimal Residual Disease Testing for Cancer	A58468 Billing and Coding: MolDX: Minimal Residual Disease Testing for Solid Tumor Cancers	Part A and B MAC	WPS*
		A59004 Billing and Coding: MolDX: Minimal Residual Disease Testing for Hematologic Cancers		
N/A	L39389 MolDX: Molecular Assays for the Diagnosis of Cutaneous Melanoma **Effective 08/06/2023**	A59163 Billing and Coding: MolDX: Molecular Assays for the Diagnosis of Cutaneous Melanoma **Effective 08/06/2023**	Part A and B MAC	CGS
N/A	L39373 MolDX: Molecular Assays for the Diagnosis of Cutaneous Melanoma **Effective 08/06/2023**	A59179 Billing and Coding: MolDX: Molecular Assays for the Diagnosis of Cutaneous Melanoma **Effective 08/06/2023**	Part A and B MAC	Noridian
N/A	L39375 MolDX: Molecular Assays for the Diagnosis of Cutaneous Melanoma **Effective 08/06/2023**	A59181 Billing and Coding: MolDX: Molecular Assays for the Diagnosis of Cutaneous Melanoma **Effective 08/06/2023**	Part A and B MAC	Noridian

NCD	LCD	LCA	Contractor Type	Contractor Name
N/A	L39345 MolDX: Molecular Assays for the Diagnosis of Cutaneous Melanoma **Effective 08/06/2023**	A59109 Billing and Coding: MolDX: Molecular Assays for the Diagnosis of Cutaneous Melanoma **Effective 08/06/2023**	Part A and B MAC	Palmetto**
N/A	L39479 MolDX: Molecular Assays for the Diagnosis of Cutaneous Melanoma **Effective 08/13/2023**	A59261 Billing and Coding: MolDX: Molecular Assays for the Diagnosis of Cutaneous Melanoma **Effective 08/13/2023**	Part A and B MAC	WPS*
N/A	L39585 MolDX: Molecular Biomarker Testing for Risk Stratification of Cutaneous Squamous Cell Carcinoma	A59382 Billing and Coding: MolDX: Molecular Biomarker Testing for Risk Stratification of Cutaneous Squamous Cell Carcinoma	Part A and B MAC	CGS
N/A	L39589 MolDX: Molecular Biomarker Testing for Risk Stratification of Cutaneous Squamous Cell Carcinoma	A59386 Billing and Coding: MolDX: Molecular Biomarker Testing for Risk Stratification of Cutaneous Squamous Cell Carcinoma	Part A and B MAC	Noridian
N/A	L39594 MolDX: Molecular Biomarker Testing for Risk Stratification of Cutaneous Squamous Cell Carcinoma	A59401 Billing and Coding: MolDX: Molecular Biomarker Testing for Risk Stratification of Cutaneous Squamous Cell Carcinoma	Part A and B MAC	Noridian
N/A	L39583 MolDX: Molecular Biomarker Testing for Risk Stratification of Cutaneous Squamous Cell Carcinoma	A59380 Billing and Coding: MolDX: Molecular Biomarker Testing for Risk Stratification of Cutaneous Squamous Cell Carcinoma	Part A and B MAC	Palmetto**
N/A	L39614 MolDX: Molecular Biomarker Testing for Risk Stratification of Cutaneous Squamous Cell Carcinoma	A59429 Billing and Coding: MolDX: Molecular Biomarker Testing for Risk Stratification of Cutaneous Squamous Cell Carcinoma	Part A and B MAC	WPS*
N/A	L39658 MolDX: Molecular Biomarkers for Risk Stratification of Indeterminate Pulmonary Nodules Following Bronchoscopy **Effective 10/13/2024**	A59482 Billing and Coding: MolDX: Molecular Biomarkers for Risk Stratification of Indeterminate Pulmonary Nodules Following Bronchoscopy **Effective 10/13/2024**	Part A and B MAC	CGS
N/A	L39678 MolDX: Molecular Biomarkers for Risk Stratification of Indeterminate Pulmonary Nodules Following Bronchoscopy **Effective 09/22/2024**	A59505 Billing and Coding: MolDX: Molecular Biomarkers for Risk Stratification of Indeterminate Pulmonary Nodules Following Bronchoscopy **Effective 09/22/2024**	Part A and B MAC	Noridian

NCD	LCD	LCA	Contractor Type	Contractor Name
N/A	L39680 MolDX: Molecular Biomarkers for Risk Stratification of Indeterminate Pulmonary Nodules Following Bronchoscopy **Effective 09/22/2024**	A59507 Billing and Coding: MolDX: Molecular Biomarkers for Risk Stratification of Indeterminate Pulmonary Nodules Following Bronchoscopy **Effective 09/22/2024**	Part A and B MAC	Noridian
N/A	L39654 MolDX: Molecular Biomarkers for Risk Stratification of Indeterminate Pulmonary Nodules Following Bronchoscopy **Effective 09/22/2024**	A59476 Billing and Coding: MolDX: Molecular Biomarkers for Risk Stratification of Indeterminate Pulmonary Nodules Following Bronchoscopy **Effective 09/22/2024**	Part A and B MAC	Palmetto**
N/A	L39711 MolDX: Molecular Biomarkers for Risk Stratification of Indeterminate Pulmonary Nodules Following Bronchoscopy **Effective 09/22/2024**	A59551 Billing and Coding: MolDX: Molecular Biomarkers for Risk Stratification of Indeterminate Pulmonary Nodules Following Bronchoscopy **Effective 09/22/2024**	Part A and B MAC	WPS*
N/A	L39650 MolDX: Molecular Testing for Risk Stratification of Thyroid Nodules **Effective 10/13/2024**	A59474 Billing and Coding: MolDX: Molecular Testing for Risk Stratification of Thyroid Nodules **Effective 10/13/2024**	Part A and B MAC	CGS
N/A	L39682 MolDX: Molecular Testing for Risk Stratification of Thyroid Nodules **Effective 07/28/2024**	A59509 Billing and Coding: MolDX: Molecular Testing for Risk Stratification of Thyroid Nodules **Effective 07/28/2024**	Part A and B MAC	Noridian
N/A	L39684 MolDX: Molecular Testing for Risk Stratification of Thyroid Nodules **Effective 07/28/2024**	A59511 Billing and Coding: MolDX: Molecular Testing for Risk Stratification of Thyroid Nodules **Effective 07/28/2024**	Part A and B MAC	Noridian
N/A	L39646 MolDX: Molecular Testing for Risk Stratification of Thyroid Nodules **Effective 07/28/2024**	A59470 Billing and Coding: MolDX: Molecular Testing for Risk Stratification of Thyroid Nodules **Effective 07/28/2024**	Part A and B MAC	Palmetto**
N/A	L39720 MolDX: Molecular Testing for Risk Stratification of Thyroid Nodules **Effective 07/28/2024**	A59560 Billing and Coding: MolDX: Molecular Testing for Risk Stratification of Thyroid Nodules **Effective 07/28/2024**	Part A and B MAC	WPS*

NCD	LCD	LCA	Contractor Type	Contractor Name
N/A	L38582 MolDX: Molecular Testing for Solid Organ Allograft Rejection	A58061 Billing and Coding: MolDX: Molecular Testing for Solid Organ Allograft Rejection	Part A and B MAC	CGS
N/A	L38629 MolDX: Molecular Testing for Solid Organ Allograft Rejection	A58168 Billing and Coding: MolDX: Molecular Testing for Solid Organ Allograft Rejection	Part A and B MAC	Noridian
N/A	L38671 MolDX: Molecular Testing for Solid Organ Allograft Rejection	A58170 Billing and Coding: MolDX: Molecular Testing for Solid Organ Allograft Rejection	Part A and B MAC	Noridian
N/A	L38568 MolDX: Molecular Testing for Solid Organ Allograft Rejection	A58019 Billing and Coding: MolDX: Molecular Testing for Solid Organ Allograft Rejection	Part A and B MAC	Palmetto**
N/A	L38680 MolDX: Molecular Testing for Solid Organ Allograft Rejection	A58207 Billing and Coding: MolDX: Molecular Testing for Solid Organ Allograft Rejection	Part A and B MAC	WPS*
N/A	L38067 MolDX: Next-Generation Sequencing for Solid Tumors	A57870 Billing and Coding: MolDX: Next-Generation Sequencing for Solid Tumors	Part A and B MAC	CGS
N/A	L38121 MolDX: Next-Generation Sequencing for Solid Tumors	A57905 Billing and Coding: MolDX: Next-Generation Sequencing for Solid Tumors	Part A and B MAC	Noridian
N/A	L38119 MolDX: Next-Generation Sequencing for Solid Tumors	A57901 Billing and Coding: MolDX: Next-Generation Sequencing for Solid Tumors	Part A and B MAC	Noridian
N/A	L38045 MolDX: Next-Generation Sequencing for Solid Tumors	A57831 Billing and Coding: MolDX: Next-Generation Sequencing for Solid Tumors	Part A and B MAC	Palmetto**
N/A	L38158 MolDX: Next Generation Sequencing for Solid Tumors	A57858 Billing and Coding: MolDX: Next-Generation Sequencing for Solid Tumors	Part A and B MAC	WPS*
N/A	L38070 MolDX: Next Generation Sequencing Lab-Developed Tests for Myeloid Malignancies and Suspected Myeloid Malignancies	A57873 Billing and Coding: MolDX: Next-Generation Sequencing Lab-Developed Tests for Myeloid Malignancies and Suspected Myeloid Malignancies	Part A and B MAC	CGS
N/A	L38123 MolDX: Next-Generation Sequencing Lab-Developed Tests for Myeloid Malignancies and Suspected Myeloid Malignancies	A57891 Billing and Coding: MolDX: Next-Generation Sequencing Lab-Developed Tests for Myeloid Malignancies and Suspected Myeloid Malignancies	Part A and B MAC	Noridian

NCD	LCD	LCA	Contractor Type	Contractor Name
N/A	L38125 MolDX: Next-Generation Sequencing Lab-Developed Tests for Myeloid Malignancies and Suspected Myeloid Malignancies	A57892 Billing and Coding: MolDX: Next-Generation Sequencing Lab-Developed Tests for Myeloid Malignancies and Suspected Myeloid Malignancies	Part A and B MAC	Noridian
N/A	L38047 MolDX: Next-Generation Sequencing Lab-Developed Tests for Myeloid Malignancies and Suspected Myeloid Malignancies	A57837 Billing and Coding: MolDX: Next-Generation Sequencing Lab-Developed Tests for Myeloid Malignancies and Suspected Myeloid Malignancies	Part A and B MAC	Palmetto**
N/A	L38176 MolDX: Next-Generation Sequencing Lab-Developed Tests for Myeloid Malignancies and Suspected Myeloid Malignancies	A57878 Billing and Coding: MolDX: Next-Generation Sequencing Lab-Developed Tests for Myeloid Malignancies and Suspected Myeloid Malignancies	Part A and B MAC	WPS*
N/A	L38394 MolDX: Pharmacogenomics Testing	A58324 Billing and Coding: MolDX: Pharmacogenomics Testing	Part A and B MAC	CGS
N/A	L38335 MolDX: Pharmacogenomics Testing	A57384 Billing and Coding: MolDX: Pharmacogenomics Testing	Part A and B MAC	Noridian
N/A	L38337 MolDX: Pharmacogenomics Testing	A57385 Billing and Coding: MolDX: Pharmacogenomics Testing	Part A and B MAC	Noridian
N/A	L38294 MolDX: Pharmacogenomics Testing	A58318 Billing and Coding: MolDX: Pharmacogenomics Testing	Part A and B MAC	Palmetto**
N/A	L38435 MolDX: Pharmacogenomics Testing	A58395 Billing and Coding: MolDX: Pharmacogenomics Testing	Part A and B MAC	WPS*
N/A	L39073 Pharmacogenomics Testing	A58812 Billing and Coding: Pharmacogenomics Testing	Part A and B MAC	First Coast
N/A	L39063 Pharmacogenomics Testing	A58801 Billing and Coding: Pharmacogenomics Testing	Part A and B MAC	Novitas**
N/A	L38584 MolDX: Phenotypic Biomarker Detection from Circulating Tumor Cells	A58063 Billing and Coding: MolDX: Phenotypic Biomarker Detection from Circulating Tumor Cells	Part A and B MAC	CGS

NCD	LCD	LCA	Contractor Type	Contractor Name
N/A	L38643 MolDX: Phenotypic Biomarker Detection from Circulating Tumor Cells	A58183 Billing and Coding: MolDX: Phenotypic Biomarker Detection from Circulating Tumor Cells	Part A and B MAC	Noridian
N/A	L38645 MolDX: Phenotypic Biomarker Detection from Circulating Tumor Cells	A58185 Billing and Coding: MolDX: Phenotypic Biomarker Detection from Circulating Tumor Cells	Part A and B MAC	Noridian
N/A	L38566 MolDX: Phenotypic Biomarker Detection from Circulating Tumor Cells	A58021 Billing and Coding: MolDX: Phenotypic Biomarker Detection from Circulating Tumor Cells	Part A and B MAC	Palmetto**
N/A	L38678 MolDX: Phenotypic Biomarker Detection from Circulating Tumor Cells	A58205 Billing and Coding: MolDX: Phenotypic Biomarker Detection from Circulating Tumor Cells	Part A and B MAC	WPS*
N/A	L38065 MolDX: Plasma-Based Genomic Profiling in Solid Tumors	A57917 Billing and Coding: MolDX: Plasma-Based Genomic Profiling in Solid Tumors	Part A and B MAC	CGS
N/A	L39230 MolDX: Plasma-Based Genomic Profiling in Solid Tumors	A58973 Billing and Coding: MolDX: Plasma-Based Genomic Profiling in Solid Tumors	Part A and B MAC	Noridian
N/A	L39232 MolDX: Plasma-Based Genomic Profiling in Solid Tumors	A58975 Billing and Coding: MolDX: Plasma-Based Genomic Profiling in Solid Tumors	Part A and B MAC	Noridian
N/A	L38043 MolDX: Plasma-Based Genomic Profiling in Solid Tumors	A57867 Billing and Coding: MolDX: Plasma-Based Genomic Profiling in Solid Tumors	Part A and B MAC	Palmetto**
N/A	L38168 MolDX: Plasma-Based Genomic Profiling in Solid Tumors	A57936 Billing and Coding: MolDX: Plasma-Based Genomic Profiling in Solid Tumors	Part A and B MAC	WPS*
N/A	L38284 MolDX: Predictive Classifiers for Early Stage Non-small Cell Lung Cancer	A58038 Billing and Coding: MolDX: Predictive Classifiers for Early Stage Non-Small Cell Lung Cancer	Part A and B MAC	CGS
N/A	L38327 MolDX: Predictive Classifiers for Early Stage Non-Small Cell Lung Cancer	A57329 Billing and Coding: MolDX: Predictive Classifiers for Early Stage Non-Small Cell Lung Cancer	Part A and B MAC	Noridian
N/A	L38329 MolDX: Predictive Classifiers for Early Stage Non-Small Cell Lung Cancer	A57330 Billing and Coding: MolDX: Predictive Classifiers for Early Stage Non-Small Cell Lung Cancer	Part A and B MAC	Noridian

NCD	LCD	LCA	Contractor Type	Contractor Name
N/A	L38238 MolDX: Predictive Classifiers for Early Stage Non-Small Cell Lung Cancer	A58031 Billing and Coding: MolDX: Predictive Classifiers for Early Stage Non-Small Cell Lung Cancer	Part A and B MAC	Palmetto**
N/A	L38443 MolDX: Predictive Classifiers for Early Stage Non-Small Cell Lung Cancer	A57112 Billing and Coding: MolDX: Predictive Classifiers for Early Stage Non-Small Cell Lung Cancer	Part A and B MAC	WPS*
N/A	L38586 MolDX: Prognostic and Predictive Molecular Classifiers for Bladder Cancer	A58065 Billing and Coding: MolDX: Prognostic and Predictive Molecular Classifiers for Bladder Cancer	Part A and B MAC	CGS
N/A	L38647 MolDX: Prognostic and Predictive Molecular Classifiers for Bladder Cancer	A58181 Billing and Coding: MolDX: Prognostic and Predictive Molecular Classifiers for Bladder Cancer	Part A and B MAC	Noridian
N/A	L38649 MolDX: Prognostic and Predictive Molecular Classifiers for Bladder Cancer	A58187 Billing and Coding: MolDX: Prognostic and Predictive Molecular Classifiers for Bladder Cancer	Part A and B MAC	Noridian
N/A	L38576 MolDX: Prognostic and Predictive Molecular Classifiers for Bladder Cancer	A58028 Billing and Coding: MolDX: Prognostic and Predictive Molecular Classifiers for Bladder Cancer	Part A and B MAC	Palmetto**
N/A	L38684 MolDX: Prognostic and Predictive Molecular Classifiers for Bladder Cancer	A58211 Billing and Coding: MolDX: Prognostic and Predictive Molecular Classifiers for Bladder Cancer	Part A and B MAC	WPS*
N/A	L37352 MolDX: Prometheus IBD sgi Diagnostic Policy	A56940 Billing and Coding: MolDX: Prometheus IBD sgi Diagnostic Policy	Part A and B MAC	CGS
N/A	L37299 MolDX: Prometheus IBD sgi Diagnostic® Policy	A57516 Billing and Coding: MolDX: Prometheus IBD sgi Diagnostic Policy	Part A and B MAC	Noridian
N/A	L37313 MolDX: Prometheus IBD sgi Diagnostic® Policy	A57517 Billing and Coding: MolDX: Prometheus IBD sgi Diagnostic Policy	Part A and B MAC	Noridian
N/A	L37260 MolDX: Prometheus IBD sgi Diagnostic® Policy	A56933 Billing and Coding: MolDX: Prometheus® IBD sgi Diagnostic Policy	Part A and B MAC	Palmetto**
N/A	L37539 MolDX: Prometheus IBD sgi Diagnostic® Policy	A57588 Billing and Coding: MolDX: Prometheus® IBD sgi Diagnostic® Policy	Part A and B MAC	WPS*

NCD	LCD	LCA	Contractor Type	Contractor Name
N/A	L36021 MolDX: Molecular Diagnostic Tests (MDT)	A54283 Billing and Coding: MolDX: SULT4A1 Genetic Testing	Part A and B MAC	CGS
N/A	N/A	A55596 Billing and Coding: MolDX: SULT4A1 Genetic Testing	Part A and B MAC	Noridian
N/A	N/A	A55601 Billing and Coding: MolDX: SULT4A1 Genetic Testing	Part A and B MAC	Noridian
N/A	L35025 MolDX: Molecular Diagnostic Tests (MDT)	A53538 Billing and Coding: MolDX: SULT4A1 Genetic Testing	Part A and B MAC	Palmetto**
N/A	L36807 MolDX: Molecular Diagnostic Tests (MDT)	A55210 Billing and Coding: MolDX: SULT4A1 Genetic Testing	Part A and B MAC	WPS*
N/A	L36021 MolDX: Molecular Diagnostic Tests (MDT)	A54901 Billing and Coding: MolDX: Targeted and Comprehensive Genomic Profile Next Generation Sequencing Testing in Cancer	Part A and B MAC	CGS
N/A	L38119 MolDX: Next-Generation Sequencing for Solid Tumors L38123 MolDX: Next-Generation Sequencing Lab-Developed Tests for Myeloid Malignancies and Suspected Myeloid Malignancies	A55624 Billing and Coding: MolDX: Targeted and Comprehensive Genomic Profile Testing in Cancer	Part A and B MAC	Noridian
N/A	L38121 MolDX: Next-Generation Sequencing for Solid Tumors L38125 MolDX: Next-Generation Sequencing Lab-Developed Tests for Myeloid Malignancies and Suspected Myeloid Malignancies	A56518 Billing and Coding: MolDX: Targeted and Comprehensive Genomic Profile Testing in Cancer	Part A and B MAC	Noridian
N/A	L38045 MolDX: Next-Generation Sequencing for Solid Tumors L38047 MolDX: Next-Generation Sequencing Lab-Developed Tests for Myeloid Malignancies and Suspected Myeloid Malignancies	A54795 Billing and Coding: MolDX: Targeted and Comprehensive Genomic Profile Testing in Cancer	Part A and B MAC	Palmetto**
N/A	L38158 MolDX: Next-Generation Sequencing for Solid Tumors	A55197 Billing and Coding: MolDX: Targeted and Comprehensive	Part A and B MAC	WPS*

NCD	LCD	LCA	Contractor Type	Contractor Name
	L38176 MolDX: Next-Generation Sequencing Lab-Developed Tests for Myeloid Malignancies and Suspected Myeloid Malignancies	Genomic Profile Testing in Cancer		
N/A	L35000 Molecular Pathology Procedures	A56199 Billing and Coding: Molecular Pathology Procedures	Part A and B MAC	NGS
N/A	L36675 ProMark® Risk Score	A57034 Billing and Coding: ProMark® Risk Score	Part A and B MAC	CGS
N/A	L36704 ProMark® Risk Score	A57515 Billing and Coding: ProMark® Risk Score	Part A and B MAC	Noridian
N/A	L36706 ProMark® Risk Score	A57609 Billing and Coding: ProMark® Risk Score	Part A and B MAC	Noridian
N/A	L36665 ProMark® Risk Score	A56957 Billing and Coding: ProMark® Risk Score	Part A and B MAC	Palmetto**
N/A	L37011 ProMark® Risk Score	A57587 Billing and Coding: ProMark® Risk Score	Part A and B MAC	WPS*

Medicare Administrative Contractor (MAC) With Corresponding States/Territories	
MAC Name (Abbreviation)	**States/Territories**
CGS Administrators, LLC (CGS)	KY, OH
First Coast Service Options, Inc. (First Coast)	FL, PR, VI
National Government Services, Inc. (NGS)	CT, IL, ME, MA, MN, NH, NY, RI, VT, WI
Noridian Healthcare Solutions, LLC (Noridian)	AS, AK, AZ, CA, GU, HI, ID, MT, NV, ND, Northern Mariana Islands, OR, SD, UT, WA, WY
Novitas Solutions, Inc. (Novitas)	AR, CO, DC, DE, LA, MD, MS, NJ, NM, OK, PA, TX, VA**
Palmetto GBA (Palmetto)	AL, GA, NC, SC, TN, VA**, WV
Wisconsin Physicians Service Insurance Corporation (WPS)*	IA, IN, KS, MI, MO, NE
Notes	
*Wisconsin Physicians Service Insurance Corporation: Contract Number 05901 applies only to WPS Legacy Mutual of Omaha MAC A Providers. **For the state of Virginia: Part B services for the city of Alexandria and the counties of Arlington and Fairfax are excluded for the Palmetto GBA jurisdiction and included within the Novitas Solutions, Inc. jurisdiction.	

CMS Claims Processing Manual

Chapter 26; § 10.4-Items 14-33 - Provider of Service or Supplier Information Instructions for Not Otherwise Classified (NOC) Codes

CMS Transmittal(s)

Transmittal 11398, Change Request 12737, Dated May 4, 2022 (Quarterly Update for Clinical Laboratory Fee Schedule (CLFS) and Laboratory Services Subject to Reasonable Charge Payment)
Transmittal 11453, Change Request 12124, Dated June 10, 2022 (International Classification of Diseases, 10th Revision (ICD-10) and Other Coding Revisions to National Coverage Determination (NCDs)--July 2021)
Transmittal 11460, Change Request 12705, Dated June 17, 2022 (International Classification of Diseases, 10th Revision (ICD-10) and Other Coding Revisions to National Coverage Determination (NCDs)--October 2022 Update)

Transmittal 11461, Change Request 12483, Dated June 21, 2022 (National Coverage Determination (NCD) 90.2, Next Generation Sequencing (NGS))
Transmittal 12021, Change Request 13195, Dated May 4, 2023 (Quarterly Update for Clinical Laboratory Fee Schedule (CLFS) and Laboratory Services Subject to Reasonable Charge Payment)
Transmittal 12207, Change Request 13166, Dated August 11, 2023 (International Classification of Diseases, 10th Revision (ICD-10) and Other Coding Revisions to National Coverage Determinations (NCDs)--October 2023 Update)
Transmittal 12355, Change Request 13278, Dated November 9, 2023 (International Classification of Diseases, 10th Revision (ICD-10) and Other Coding Revisions to National Coverage Determinations (NCDs)--January 2024 Update)
Transmittal 12444, Change Request 13278, Dated January 4, 2023 (International Classification of Diseases, 10th Revision (ICD-10) and Other Coding Revisions to National Coverage Determinations (NCDs)--January 2024 Update)
Transmittal 12626, Change Request 13596, Dated May 9, 2024, (International Classification of Diseases, 10th Revision (ICD-10) and Other Coding Revisions to National Coverage Determinations (NCDs)--October 2024)

Other(s)

Billing and Describing Not Otherwise Classified (NOC) Codes (wpsgha.com)
CMS Clinical Laboratory Fee Schedule, CMS Website
Palmetto GBA MolDx Website
Palmetto GBA MolDx Manual, Palmetto GBA MolDx Website
L38380 MolDX: AlloSure® or Equivalent Cell-Free DNA Testing for Kidney and Heart Allografts; Retired 06/09/2023
A57233 Billing and Coding: MolDX: AlloSure® or Equivalent Cell-Free DNA Testing for Kidney and Heart Allografts; Retired 06/09/2023
L38355 MolDX: AlloSure® or Equivalent Cell-Free DNA Testing for Kidney and Heart Allografts; Retired 06/09/2023
A57380 Billing and Coding: MolDX: AlloSure® or Equivalent Cell-Free DNA Testing for Kidney and Heart Allografts; Retired 06/09/2023
L37733 Biomarker Testing for Prostate Cancer Diagnosis; Retired 03/01/2024
A56609 Billing and Coding: Biomarker Testing for Prostate Cancer Diagnosis
L36499 BRCA1 and BRCA2 Genetic Testing
A57449 Billing and Coding: BRCA1 and BRCA2 Genetic Testing
L34912 Genetic Testing for Lynch Syndrome
A57450 Billing and Coding: Genetic Testing for Lynch Syndrome
L37810 Genomic Sequence Analysis Panels in the Treatment of Solid Organ Neoplasms
A56867 Billing and Coding: Genomic Sequence Analysis Panels in the Treatment of Solid Organ Neoplasms
A55137 Billing and Coding: MolDX 4q25-AF Risk Genotype Testing; Retired 05/30/2024
A53457 Billing and Coding: MolDX: 4q25-AF Risk Genotype; Retired 04/16/2024
A55090 Billing and Coding: MolDX: 4q25-AF Risk Genotype; Retired 04/16/2024
A55091 Billing and Coding: MolDX: 4q25-AF Risk Genotype; Retired 04/16/2024
A54241 Billing and Coding: MolDX: 4q25-AF Risk Genotype Guidelines; Retired 04/16/2024
A55138 Billing and Coding: MolDX: 9p21 Genotype Test; Retired June 27, 2024
A53657 Billing and Coding: MolDX: 9p21 Genotype Test; Retired 05/24/2024
A55092 Billing and Coding: MolDX: 9p21 Genotype Test; Retired 05/24/2024
A55093 Billing and Coding: MolDX: 9p21 Genotype Test; Retired 05/24/2024
A54253 Billing and Coding: MolDX: Aspartoacyclase 2 Deficiency (ASPA) Testing; Retired 05/14/2024
A55142 Billing and Coding: MolDX: Aspartoacyclase 2 Deficiency (ASPA) Testing; Retired 06/27/2024
A53602 Billing and Coding: MolDX: Aspartoacyclase 2 Deficiency (ASPA) Testing; Retired 05/14/2024
A55088 Billing and Coding: MolDX: Aspartoacyclase 2 Deficiency (ASPA) Testing; Retired 05/14/2024
A55089 Billing and Coding: MolDX: Aspartoacyclase 2 Deficiency (ASPA) Testing; Retired 05/14/2024
A54253 Billing and Coding: MolDX: Aspartoacyclase 2 Deficiency (ASPA) Testing; Retired 05/14/2024
A55143 Billing and Coding: MolDX: ATP7B Gene Tests; Retired 05/30/2024
A53550 Billing and Coding: MolDX: ATP7B Gene Tests; Retired 04/23/2024
A55097 Billing and Coding: MolDX: ATP7B Gene Tests; Retired 04/23/2024
A55098 Billing and Coding: MolDX: ATP7B Gene Tests; Retired 04/23/2024
A54254 Billing and Coding: MolDX: ATP7B Gene Tests; Retired 04/23/2024
A53565 Billing and Coding: MolDX: CHD7 Gene Analysis; Retired 04/25/2024
A55085 Billing and Coding: MolDX: CHD7 Gene Analysis; Retired 04/25/2024
A55157 Billing and Coding: MolDX: CHD7 Gene Analysis; Retired 05/30/2024
A54243 Billing and Coding: MolDX: CHD7 Gene Analysis Guidelines; Retired 04/25/2024
A55086 Billing and Coding: MolDX: CHD7 Gene Analysis; Retired 04/25/2024
A53536 Billing and Coding: MolDX: ENG and ACVRL1 Gene Tests; Retired 04/18/2024
A54262 Billing and Coding: MolDX: ENG and ACVRL1 Gene Tests; Retired 04/18/2024
A55159 Billing and Coding: MolDX: ENG and ACVRL1 Gene Tests; Retired 05/30/2024

A55181 Billing and Coding: MolDX: ENG and ACVRL1 Gene Tests; Retired 04/18/2024
A55182 Billing and Coding: MolDX: ENG and ACVRL1 Gene Tests; Retired 04/18/2024
A53619 Billing and Coding: MolDX: HAX1 Gene Sequencing; Retired 05/14/2024
A54266 Billing and Coding: MolDX: HAX1 Gene Sequencing; Retired 05/14/2024
A55165 Billing and Coding: MolDX: HAX1 Gene Sequencing; Retired 06/27/2024
A55249 Billing and Coding: MolDX: HAX1 Gene Sequencing; Retired 05/14/2024
A55252 Billing and Coding: MolDX: HAX1 Gene Sequencing; Retired 05/14/2024
A53576 Billing and Coding: MolDX: KIF6 Genotype; Retired 04/26/2024
A55171 Billing and Coding: MolDX: KIF6 Genotype; Retired 05/30/2024
A55272 Billing and Coding: MolDX: KIF6 Genotype; Retired 04/26/2024
A55273 Billing and Coding: MolDX: KIF6 Genotype; Retired 04/26/2024
A53467 Billing and Coding: MolDX: LPA-Aspirin Genotype; Retired 04/16/2024
A54275 Billing and Coding: MolDX: LPA-Aspirin Genotype; Retired 04/16/2024
A55173 Billing and Coding: MolDX: LPA-Aspirin Genotype; Retired 05/30/2024
A55279 Billing and Coding: MolDX: LPA-Aspirin Genotype; Retired 04/16/2024
A55280 Billing and Coding: MolDX: LPA-Aspirin Genotype; Retired 04/16/2024
A53468 Billing and Coding: MolDX: LPA-Intron 25 Genotype; Retired 04/17/2024
A54276 Billing and Coding: MolDX: LPA-Intron 25 Genotype; Retired 04/17/2024
A55174 Billing and Coding: MolDX: LPA-Intron 25 Genotype; Retired 05/30/2024
A55281 Billing and Coding: MolDX: LPA-Intron 25 Genotype; Retired 04/17/2024
A55282 Billing and Coding: MolDX: LPA-Intron 25 Genotype; Retired 04/17/2024
A53574 Billing and Coding: MolDX: MECP2 Genetic; Retired 04/26/2024
A55189 Billing and Coding: MolDX: MECP2 Genetic Testing; Retired 05/30/2024
A55285 Billing and Coding: MolDX: MECP2 Genetic Testing; Retired 04/26/2024
A55286 Billing and Coding: MolDX: MECP2 Genetic Testing; Retired 04/26/2024
A56501 Billing and Coding: MolDX: Microsatellite Instability-High (MSI-H) and Mismatch Repair Deficient (dMMR) Biomarker for Patients with Unresectable or Metastatic Solid Tumors; Retired 01/26/2023
A53669 Billing and Coding: MolDX: Mitochondrial Nuclear Gene Tests; Retired 05/29/2024
A55190 Billing and Coding: MolDX: Mitochondrial Nuclear Gene Tests; Retired 06/27/2024
A55290 Billing and Coding: MolDX: Mitochondrial Nuclear Gene Tests; Retired 05/29/2024
A55291 Billing and Coding: MolDX: Mitochondrial Nuclear Gene Tests; Retired 05/29/2024
A54288 Billing and Coding: MolDX: Mitochondrial Nuclear Gene Tests Guidelines; Retired 05/29/2024
L36886 MolDX: Percepta© Bronchial Genomic Classifier; Retired 09/22/2024
L38997 MolDX: Molecular Biomarkers to Risk-Stratify Patients at Increased Risk for Prostate Cancer
A58713 Billing and Coding: MolDX: Molecular Biomarkers to Risk-Stratify Patients at Increased Risk for Prostate Cancer
L39005 MolDX: Molecular Biomarkers to Risk-Stratify Patients at Increased Risk for Prostate Cancer
A58718 Billing and Coding: MolDX: Molecular Biomarkers to Risk-Stratify Patients at Increased Risk for Prostate Cancer
L39007 MolDX: Molecular Biomarkers to Risk-Stratify Patients at Increased Risk for Prostate Cancer
A58724 Billing and Coding: MolDX: Molecular Biomarkers to Risk-Stratify Patients at Increased Risk for Prostate Cancer
L38985 MolDX: Molecular Biomarkers to Risk-Stratify Patients at Increased Risk for Prostate Cancer
A58700 Billing and Coding: MolDX: Molecular Biomarkers to Risk-Stratify Patients at Increased Risk for Prostate Cancer
L39042 MolDX: Molecular Biomarkers to Risk-Stratify Patients at Increased Risk for Prostate Cancer
A58759 Billing and Coding: MolDX: Molecular Biomarkers to Risk-Stratify Patients at Increased Risk for Prostate Cancer
L35025 MolDX: Molecular Diagnostic Tests (MDT)
A56973 Billing and Coding: MolDX: Molecular Diagnostic Tests (MDT)
L35160 MolDX: Molecular Diagnostic Tests (MDT)
A57526 Billing and Coding: MolDX: Molecular Diagnostic Tests (MDT)
L36256 MolDX: Molecular Diagnostic Tests (MDT)
A57527 Billing and Coding: MolDX: Molecular Diagnostic Tests (MDT)
L35025 MolDX: Molecular Diagnostic Tests (MDT)
A56853 Billing and Coding: MolDX: Molecular Diagnostic Tests (MDT)
L36807 MolDX: Molecular Diagnostic Tests (MDT)
A57772 Billing and Coding: MolDX: Molecular Diagnostic Tests (MDT)
A53585 Billing and Coding: MolDX: NSD1 Gene Tests; Retired 04/30/2024
A54291 Billing and Coding: MolDX: NSD1 Gene Tests; Retired 04/30/2024
A55198 Billing and Coding: MolDX: NSD1 Gene Tests; Retired 05/30/2024
A55609 Billing and Coding: MolDX: NSD1 Gene Tests; Retired 04/30/2024
A55615 Billing and Coding: MolDX: NSD1 Gene Tests; Retired 04/30/2024
A57502 Billing and Coding: MolDX: Percepta© Bronchial Genomic Classifier; Retired 09/22/2024
A53664 Billing and Coding: MolDX: PAX6 Gene Sequencing; Retired 05/29/2024

A55199 Billing and Coding: MolDX: PAX6 Gene Sequencing; Retired 06/27/2024
A54293 Billing and Coding: MolDX: PAX6 Gene Sequencing Guidelines; Retired 05/29/2024
A55625 Billing and Coding: MolDX: PAX6 Gene Sequencing; Retired 05/29/2024
A55632 Billing and Coding: MolDX: PAX6 Gene Sequencing; Retired 05/29/2024
L36891 MolDX: Percepta© Bronchial Genomic Classifier; Retired 09/22/2024
A57504 Billing and Coding: MolDX: Percepta© Bronchial Genomic Classifier; Retired 09/22/2024
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L36908 MolDX: Percepta© Bronchial Genomic Classifier; Retired 09/22/2024
A56972 Billing and Coding: MolDX: Percepta® Bronchial Genomic Classifier; Retired 09/22/2024
A53567 Billing and Coding: MolDX: PTCH1 Gene Testing; Retired 04/25/2024
A54297 Billing and Coding: MolDX: PTCH1 Gene Testing; Retired 04/25/2024
A55203 Billing and Coding: MolDX: PTCH1 Gene Testing; Retired 05/30/2024
A55608 Billing and Coding: MolDX: PTCH1 Gene Testing; Retired 04/25/2024
A55618 Billing and Coding: MolDX: PTCH1 Gene Testing; Retired 04/25/2024
L38288 MolDX: Repeat Germline Testing
A57141 Billing and Coding: MolDX: Repeat Germline Testing
L38351 MolDX: Repeat Germline Testing
A57331 Billing and Coding: MolDX: Repeat Germline Testing
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A57100 Billing and Coding: MolDX: Repeat Germline Testing
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A55614 Billing and Coding: MolDX: RPS19 Gene Tests; Retired 04/30/2024
A54830 Billing and Coding: MolDX: Short Tandem Repeat (STR) Markers and Chimerism (CPT® codes 81265-81268)
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A54282 Billing and Coding: MolDX: TERC Gene Tests; Retired 05/01/2024
A55211 Billing and Coding: MolDX: TERC Gene Tests; Retired 05/30/2024
A55611 Billing and Coding: MolDX: TERC Gene Tests; Retired 05/01/2024
A55611 Billing and Coding: MolDX: TERC Gene Tests; Retired 05/01/2024
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L34519 Molecular Pathology Procedures
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A59698 MolDX: Defining panel services in MolDX
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A59678 MolDX: Defining panel services in MolDX
A59700 MolDX: Defining panel services in MolDX
A55293 Billing and Coding: MolDX: myPap™; Retired 04/19/2024
A54290 Billing and Coding: MolDX: myPap™; Retired 04/19/2024
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A55275 Billing and Coding: MolDX: Know error®; Retired 04/24/2024

Clinical Evidence

Biomarkers for Oncology

MyPRS

MyPRS test is a microarray-based gene expression profiling (GEP) assay of a 70-gene signature of aggressive disease (GEP70) performed on CD138-positive (CD138 +) plasma cells derived from bone marrow aspirate. The gene expression data are used to generate a risk score from 0 (lowest risk) to 100 (highest risk). A cutoff score of 45.2 is used to distinguish between low-risk (good prognosis) and high-risk (poor prognosis) patients. Zhou et al. (2009) Microarray-based gene expression profiling (GEP) analysis estimates the underlying activity of cellular pathways in a tissue sample that control, for example, cell division or proliferation, apoptosis (programmed cell death), metabolism, or other signaling pathways. Relative over- or under expression of genes in these pathways is considered to mirror disease aggressiveness independent of cytogenetics and other laboratory measures. GEP-defined high risk was present in 52% of the pts w/ CA in randomly sampled and focal lesion bone marrow sites, and in 9% of the remainder (P < 0.001). GEP analysis has been proposed as a means to more accurately stratify MM patients into risk categories, which may inform therapy selection according to tumor biology.

Rosetta Cancer Origin Test™

Meleth et al. (2013) performed a technology assessment on genetic testing or molecular pathology testing of cancers of unknown primary (CUP) site to determine origin, focusing on analytical and clinical validity and utility in guiding the diagnosis and treatment of CUP and improving health outcomes. The authors reported on the accuracy of miRview in identifying the primary site in a tissue of unknown origin with ratings ranging from fair to good on validation studies. The summarized meta-analytic estimate across the studies was 85 percent; the evidence is high that miRview mets accurately identifies the tumor type in known tissue 85 percent of the time.

Meiri et al. (2012) reported on the development and validation of a second-generation assay identifying 42 tumor types using a microRNA-based assay. The authors reported an overall sensitivity of 85% that was measured blindly on a validation set of 509 independent samples and reported 88% clinical validation on 52 true cancers of unknown primary (CUP) patients. The authors concluded a high accuracy of the abilities of the assay to identify 42 tumor types as a reliable diagnostic tool for cancers of unknown or uncertain primary origins.

RosettaGX Reveal

Silaghi et al. (2021) conducted a systematic review and meta-analysis that addressed RosettaGX Reveal thyroid miRNAs classifier that stratifies indeterminate thyroid nodules (ITNs) by evaluating the expression of 24 up and down-regulated miRNAs species. The authors reported three studies enrolled 234 cytologically ITNs and tested them with RosettaGX Reveal molecular panel from 2015 to 2018. RosettaGX Reveal negative and positive results were nearly equal (53% vs. 47%). There was a 99% surgery rate of the nodules with a valid test result. After surgical treatment, the histological assessment revealed 72 of 120 (60%) malignant tumors. The authors concluded that the preliminary results of RosettaGX Reveal panel sensitivity ranged from 85.2 to 100% and specificity ranged from 69.2 to 85.7% across studies.

Vargas-Salas et al. (2018) performed a review and meta-analysis of genetic testing for indeterminate thyroid cytology (ITC). The RosettaGX Reveal was one of the 26 studies included in the systematic review that has reported multicentric clinical validation studies along with ThyGeNext/ThyraMIR. The authors reported that the RosettaGX Reveal cohort was predominantly composed by Bethesda IV cytologies (87%), with an overall cancer prevalence of 21% on ITC. However, information about the Bethesda III and Bethesda IV regarding specific cancer prevalence was not available since there was no information provided about the diagnostic performance. Only theoretical estimations were performed for ThyGenX/ThyraMIR and RosettaGX Reveal because there were no reported post-validation studies. RosettaGX Reveal showed a sensitivity of 74% when considering the entire cohort, and 100% when non-agreement gold standard cases were excluded. The authors concluded that the test performance could potentially avoid 74% of surgeries for benign nodules.

Uveal Melanoma GNA11

Uveal melanoma (UM) is the most common primary intraocular cancer in adults, most of which can be treated and locally controlled with radiation therapy or resection. However, up to 50% of those with UM develop metastases that are fatal within one year. Most UMs are triggered by a mutation in the GNAQ or GNA11 (Sorrentino, 2024). Forty-nine individuals with UM were included in a study performed by Schneider et al. (2019) designed to analyze GNAQ and GNA11 mutations using formalin-fixed, paraffin-embedded (FFPE) samples and compare the results with clinicopathological parameters. High frequencies of mutations in GNAQ and GNA11 mutations were detected with about a fourth of cases demonstrating not yet reported mutations. The review notes that since recent studies have shown mutations in GNAQ and GNA11 are sensitive to MAP kinase, protein kinase C, AKT and YAP inhibitors there may be a therapeutic option for metastasized tumors; additionally, the analysis of GNAQ and GNA11 mutations may become a routine diagnostic procedure of UM.

CIMP

Gu et al. (2023) stated that CpG island methylator phenotype (CIMP), characterized by the concurrent and widespread hypermethylation of a cluster of CpGs, has been reported to play an important role in carcinogenesis. In their study to identify two subtypes that displayed markedly distinct DNA methylation levels, termed CIMP (high levels of DNA methylation) and nCIMP subgroup (low levels of DNA methylation), the authors studied the role of CIMP in papillary thyroid carcinomas (PTCs). The study included a genome-wide DNA methylation analysis of 350 primary PTCs from the Cancer Genome Atlas database with 57 patients with CIMP and 293 patients with nCIMP. The authors reported that differential methylation analysis showed a broad methylation gain in CIMP, and that generalized gene set testing analysis showed remarkable enrichment in epithelial mesenchymal transition and angiogenesis hallmark pathways, and that the CIMP phenotype may promote the tumor progression from another perspective. The authors also reported that their analysis of tumor microenvironment showed that CIMP PTCs are in an immune-depletion status, which may affect the effectiveness of immunotherapy. The authors concluded that PTC patients with CIMP appeared to have a higher degree of malignancy, as the patients were older with advanced pathological stage, and lymph node metastasis and they showed remarkable poor clinical outcomes when they were compared to patients with nCIMP regarding overall survival and progression-free survival. The authors also concluded that CIMP was associated with worse survival independent of known prognostic factors. Limitations of the study include the small sample size and the retrospective design.

Padmanabhan et al. (2021) conducted a study to discover molecular contributors to CIMP in gastric cancer (GC), by performing global DNA methylation, gene expression, and proteomics profiling across 14 gastric cell lines, followed by similar integrative analysis in 50 GC cell lines and 467 primary GCs. The authors assembled a panel of 14 gastric cell lines based on previous literature that comprised three gastric/ microsatellite instability (MSI) CIMP (NUGC3, NCC59, and IM95), three Epstein-Barr Virus (EBV)-CIMP (SNU719, YCC10 and NCC24), six non-CIMP GC cell lines (SNU16, SNU484, SNU1967, SNU1750, NCC19 and MKN1), and two non-malignant gastric epithelial cell lines (GES1 and HFE145). The authors acquired GC patient samples for quantitative PCR validation from the SingHealth tissue repository and performed global methylation sequencing, RNA sequencing, and proteomic analysis on the 14-cell line panel. The authors reported that the cystathionine beta-synthase enzyme (CBS) was identified as a highly recurrent target of epigenetic silencing in CIMP GC and that CBS epimutations were significantly associated with CIMP in various other cancers that occur in premalignant gastroesophageal conditions and was longitudinally linked to clinical persistence. The authors concluded that CBS is a bi-faceted modifier of aberrant DNA methylation and inflammation in GC, and that CBS highlighted hydrogen sulfide donors as potential new therapy for CBS-silenced lesions. This study was limited by the retrospective design.

PTEN

Yehia et al. (2020) performed literature review. The authors concluded that the PTEN mutation landscape cannot independently account for the variability in observed phenotypes. This poses a clinical challenge, as individuals carrying identical PTEN mutations may have completely different disease manifestations and natural histories. Intriguingly, burgeoning data indicate that genetic and nongenetic modifiers may be key contributors dictating specific clinical phenotypes in PHTS. Ongoing research and clinical trials preface an era of predictive medicine, whereby treatment is not only preventive but also specific to the individual. One major question that remains is how to translate the predictive value of such PTEN-modifier combinations into the clinic. Systematic and longitudinal studies will be fundamental for unraveling the complexity of PTEN-modifier–phenotype interactions.

AKT1

In a 2020 review article, Chen et al. described how AKT1(E17K) mutations stimulate downstream signals that cause cells to transform and explore the differential regulation and function of E17K in different physiological and pathological settings. The authors also describe the phenomenon that E17K impedes tumor growth by interfering with growth-promoting and chemotherapy-resistant AKT1lowQCC. It was concluded that AKT1 is a key mediator of signaling pathways involved in cell survival, proliferation and growth. The AKT1(E17K) mutation activates the PI3K-AKT pathway to

enhance cell migration and oncogenic capacity. Conversely, E17K expression may be involved in the regression process of solid tumors and may be shown to help stop the growth of human solid tumors. This review offers new complexity to the role of this hotspot mutation in cancer progression and raises the possibility of treating cancer by targeting the mutant AKT1.

In a 2018 study, Chang et al. analyzed 24,592 cancers including 10,336 prospectively sequenced patients with advanced disease to identify mutant residues arising more frequently than expected in the absence of selection. The authors identified 1,165 statistically significant hotspot mutations of which 80% arose in 1 in 1000 or fewer patients. Of 55 recurrent in-frame indels, they validated that novel AKT1 duplications induced pathway hyperactivation and conferred AKT inhibitor sensitivity. Consequently, 26% of all hotspots in therapeutically actionable oncogenes were novel. Upon matching a subset of affected patients directly to molecularly targeted therapy, we observed radiographic and clinical responses. Population-scale mutant allele discovery illustrates how the identification of driver mutations in cancer is far from complete. Our systematic computational, experimental, and clinical analysis of hotspot mutations in almost 25,000 human cancers demonstrates that the long right tail of biologically and therapeutically significant mutant alleles is still incompletely characterized. Sharing prospective genomic data will accelerate hotspot identification expanding the reach of precision oncology in cancer patients.

RB1

Plimack et al. (2024) analyzed 105 tumor specimens from patients who participated in a large, multi-center trial and had been diagnosed with muscle-invasive bladder cancer (MIBC) but had not yet undergone neoadjuvant chemotherapy (NAC) followed by cystectomy. The study population was 90% white, 85% male with a median age of 65 years and with a genetic variant of one of the four genes in 53% of cases. The authors reported that patients with tumors with a mutation in ATM, RB1, or ERCC2 had significantly higher odds of achieving cancer-free surgical specimens at the time of cystectomy (pT0) and that, if the three-gene test is negative for pT0 (i.e., no mutation in ATM, ERCC2, or RB1), the disease is likely to be present. The authors also stated that there was a significant correlation between the number of mutations and pT0 as 14% of patients had no mutations, 41% had one mutation, and 67% had two or more mutations. The authors also reported that the biomarker was better at predicting the presence of disease than the absence of disease and that there was no evidence of in interaction between the types of chemotherapy used. Limitations of the study include the small sample size and the lack of long term follow-up. The authors concluded that their analysis of pretreatment tumor DNA for patients with MIBC validated the relationship of the three-gene biomarker with pathological outcomes and its ability to select patients for bladder preservation.

In a 2022 review article, Yao et al. focused on the RB1 mutation and oncogenesis. While a mutation in the RB1 gene is seen in almost every case of retinoblastoma, alterations also occur in multiple types of cancer. There are vast reports of mutation data with approximately 2000 variants identified. RB1 functions as a chromatin-associated protein and regulates the cell cycle, and the current understanding of RB1 includes other activities such as genomic instability, apoptosis, and metabolic reprogramming. RB1 mutations have been known for being unresponsive to drugs which has limited breakthrough treatments for related cancers. The relatively high frequency and severe effects of RB1 mutation also support its clinical application as a biomarker. Future high quality research on RB1 mutations should include the type of mutations amenable to therapy.

Manzano et al. (2021) conducted a study to evaluate whether the presence of somatic co-alterations in RB1 and TP53 in muscle invasive urothelial bladder carcinoma (MIBC) affects their responsiveness to immunity checkpoint inhibitors (ICIs). The study included the urothelial bladder cancer dataset from 407 patients in the Cancer Genome Atlas (TCGA) who had mutational, copy number and transcriptomic data. The authors found that 187 (45.9%) of the patients had no genomic alterations for either TP53 or RB1, 30 patients (7.4%) had RB1 genomic alterations only, 121 (29.7%) had TP53 genomic alterations, and 69 (17.0%) had genomic alterations in the RB1 and TP53 genes concurrently. The authors reported that patients with genomic alterations in RB1 only did not show significant differences in any of the 12 scores tested (10 cell populations and two immunological signatures) when compared to the patients with no genomic alterations for either RB1 or TP53, while those with alterations in TP53 only showed significant differences in the endothelial cells score when compared to the patients with no genomic alterations. The authors also reported that patients with alterations in both RB1 and TP53 showed the largest differences in three cell populations (significantly higher scores in cytotoxic lymphocytes and NK cells, and significantly lower scores in endothelial cells) when compared to patients with no genomic alterations for either RB1 or TP53. Finally, the authors reported that the T cell inflamed signature had a significantly higher score in patients with alterations in both RB1 and TP53 compared to patients with no alterations for either gene. Limitations of the study include its retrospective nature and the limited number of RB1 and TP53 mutants available. The authors concluded that they found compelling evidence regarding the association of concurrent genomic alterations in RB1 and TP53 with factors related to favorable response to treatment with ICIs.

George et al. (2015) conducted a study on complex genomic rearrangements, which are undetectable by exome sequencing, to determine if they further contribute to the pathogenesis of small cell lung cancer (SCLC). The authors collected 152 fresh-frozen tumor specimens from patients diagnosed with stage I-IV SCLC. The specimens included 148 primary lung samples, and four metastatic samples obtained by surgical resection (n = 132), biopsy (n = 4), pleural effusion (n = 1) or through autopsy (n = 15). The authors performed whole-genome sequencing on 110 of the tumors and their matched normal DNA. They excluded 42 cases from the analysis due to insufficient quality or amount of DNA. The authors stated that most of the 110 tumors were treatment-naive, with only five cases acquired at the time of relapse. The authors analyzed transcriptome sequencing data in 71 of the 110 specimens that had undergone genome sequencing and in 10 additional specimens. The authors reported that inactivating mutations were found in nearly all of the tumors they analyzed with mutations present in 90% of TP53 and 65% of RB1 in patients with SCLC, and that smoking history or clinical stage of the tumors did not correlate with the type and number of mutations. The authors conclude that their study provided a comprehensive genomic analysis of SCLC that implicated several previously unknown genes and biological processes in the pathogenesis of SCLC as possible targets for more effective targeted therapeutic interventions against the disease. The study is limited by the small sample size.

MLL/AF4

In their systematic review and meta-analysis, Ye et al. (2023) that evaluated whether lysine (K) methyltransferase 2A (KMT2A), also known as mixed lineage leukemia (MLL), has a prognostic impact on patients with acute myeloid leukemia (AML). The meta-analysis included 18 studies that included 17 cohort studies and one RCT, all of which were assessed to be of high quality. The studies included a total of 6499 patients (median age 60 years or older) with AML, of which 705 were partial tandem duplication of KMT2A (KMT2A-PTD) positive (10.85%). The authors reported that KMT2A-PTD conferred shorter overall survival in total population when compared with the KMT2A-PTD-negative AML patients, and the subgroup analysis showed that KMT2A-PDT also resulted in shorter overall survival in patients with karyotypically normal AML and in older patients with AML. The authors also reported that KMT2A-PTD indicated no prognostic impact on event-free survival (EFS) in the total population although, in the sensitivity analysis, KMT2A-PTD resulted in poor EFS when they deleted one study with a relatively obvious effect on the combined hazard ratio. The authors also reported that their subgroup analysis showed that KMT2A-PTD was associated with poor EFS in older patients with AML. Limitations of the study include the cohort study design of most of the included studies, the lack of availability of raw data for each individual patient, and the lack of inclusion of the potential effects of other factors (e.g., gender, chromosomal aberration, cytogenetic risk classification, gene lesions and time of follow-up). The authors concluded that KMT2A-PTD had a significantly unfavorable prognostic effect in patients with AML as well as to some subgroups, including karyotypically normal AML, older patients with AML (> 60 years old) and patients with acute promyelocytic leukemia.

Hu et al. (2021) conducted a study to evaluate AFF4 expression levels and clinicopathological features in melanoma tissue samples to investigate the mechanism of molecular metastasis. The study included 110 melanoma samples and 68 benign nevi samples from 79 patients (53.2% male, 58.2% aged sixty or over) who had been histopathologically and clinically diagnosed. The authors reported that AFF4 expression was upregulated in melanoma tumor tissues and high AFF4 expression in melanoma tissues correlated with poor survival. The authors also reported that AFF4 promoted melanoma cell proliferation with the invasion and migration of melanoma cells by mediating epithelial to mesenchymal transition (EMT), that AFF4 enhances melanoma cell migration and invasion, and that AFF4 may regulate c-Jun/MMP2 signaling in melanoma cells. The authors concluded that high AFF4 expression was correlated with poor survival of cutaneous melanoma patients and that AFF4 enhanced malignant progression through modulation of c-Jun activity which demonstrated that AFF4 acted as an aggressive factor in melanoma.

DEK/CAN

Oancea et al. (2010) conducted a study on t(6;9)-positive acute myeloid leukemia (AML), which is classified as a separate clinical entity because of its early onset and poor prognosis. The hallmark of t(6;9) AML is the expression of the DEK/CAN fusion protein. The leukemogenic potential of DEK/CAN has been called into question, because it was shown to be unable to block the differentiation of hematopoietic progenitors. The authors found that DEK/CAN initiated leukemia from a small subpopulation within the hematopoietic stem cell (HSC) population expressing a surface marker pattern of long-term (LT) HSC. The propagation of established DEK/CAN-positive leukemia was not restricted to the LT-HSC population but occurred even from more mature and heterogeneous cell populations. This finding indicates that in DEK/CAN-induced leukemia, there is a difference between 'leukemia-initiating cells' (L-ICs) and 'leukemia-maintaining cells' (L-MCs). In contrast to the L-IC cells represented by a very rare subpopulation of LT-HSC, the L-MC seem to be represented by a larger and phenotypically heterogeneous cell population.

TET2

In 2010, Tefferi et al. conducted a review to analyze the functional and clinical relevance of different genes in myeloproliferative neoplasms (MPN). With regard to TET2 mutations, the authors found that mutations in this gene are

found in many places which makes it difficult to understand the specific pathogenic contribution to MPNs. The presence of the mutant TET2 did not seem to affect survival, leukemic transformation, thrombosis risk or cytogenetic profile in either polycythemia vera or primary myelofibrosis, however it was associated with superior survival in myelodysplastic syndrome and inferior survival in acute myeloid leukemia and chronic myelomonocytic leukemia. The pervasive nature of TET2 mutations undermines their specific pathogenetic contribution to MPN.

In a 2009 study, Delhommeau et al. conducted an analysis using combination of molecular, cytogenetic, comparative-genomic-hybridization, and single-nucleotide–polymorphism to identify a candidate tumor- suppressor gene common to patients with myelodysplastic syndromes, myeloproliferative disorders, and acute myeloid leukemia (AML). Samples of bone marrow or blood were obtained from 320 patients (84 with myelodysplastic syndromes, 203 with myeloproliferative disorders, 2 with primary AML, 22 with secondary AML, and 9 with chronic myelomonocytic leukemia). The coding sequence of TET2 was determined in all samples analyzed and it was found that deletions or mutations in TET2 are early events in some patients with myelodysplastic syndromes, myeloproliferative disorders, or secondary AML.

In a 2009 study, Abdel-Wahab et al. evaluated the mutational status of TET1, TET2, and TET3 in myeloproliferative neoplasms (MPNs), chronic myelomonocytic leukemia (CMML), and acute myeloid leukemia (AML). The sequencing of TET2 in 408 paired tumor/normal samples showed 68 somatic mutations and 6 novel single nucleotide polymorphisms and identified TET2 mutations in MPN (27 of 354, 7.6%), CMML (29 of 69, 42%), AML (11 of 91, 12%), and M7 AML (1 of 28, 3.6%) samples. TET2 mutations did not cluster in genetically defined MPN, CMML, or AML subsets but were associated with decreased overall survival in AML. These results indicate that TET2 mutations are observed in different myeloid malignancies and may be important in AML prognosis.

CALR

Ha et al (2015) studied the frequency and type of Calreticulin (CALR) mutations and hematological characteristics in patients with myeloproliferative neoplasms (MPNs) in 168 MPN patients (36 polycythemia vera [PV], 114 essential thrombocythemia [ET], and 18 primary myelofibrosis [PMF] cases). CALR mutations were detected in 21.9% of ET and 16.7% of PMF patients, that accounted for 58.5% and 33.3% of ET and PMF patients without Janus kinase 2 (JAK2) or myeloproliferative leukemia virus oncogenes (MPL) mutations, respectively. A total of five types of mutation were detected, among which, L367fs*46 (53.6%) and K385fs*47 (35.7%) were found to be the most common. ET patients with CALR mutation had lower leukocyte counts and ages compared with JAK2-mutated ET patients. The authors concluded genotyping for CALR could be useful as a diagnostic tool for JAK2-or MPL-negative ET or PMF patients. CALR mutation may be a distinct disease group, with different hematological characteristics than that of JAK2-positive patients.

CSF3R

In a 2024 review article, Szuber et al. presented an overview of the diagnosis, genetics, risk stratification and management of CNL and ACML. It was reported that oncogenic driver mutations in the CSF3R gene remain the genetic signature of CNL, for which the World health Organization has included in the 2022 diagnostic criteria for this disorder. Genotype–phenotype associations are apparent in CNL with distinct clinical profiles depending on the CSF3R mutation types. aCML was verified as a genetically complex and heterogeneous disease with lower frequency of CSF3R. These insights into pathogenesis, clonal evolution, and molecular mechanisms of resistance to therapy can provide a framework for personalized management. From a management standpoint, significant challenges remain, including translating genomic data into accurate and clinically applicable prognostic and treatment paradigms. Moreover, novel drug targets and drug combinations must be explored with the objective of eradicating mutant clones. Determining optimal candidates for, and timing of HSCT, as well as clarifying the role of peri-transplant therapy, are also issues that need to be formally addressed.

TSC2

In a 2022 study, Huang et al. clarified the mutation landscape and potential correlation between sporadic lymphangioleiomyomatosis (S-LAM) genomic profiles and clinical phenotypes. Genomic profiles of 22 S-LAM patients were obtained by sequencing genomic and cell-free DNA from various specimens using a next-generation sequencing (NGS)-based tumor-driver gene panel. Symptoms, serum vascular endothelial growth factor D (VEGF-D) values, pulmonary function, and six-minute walk distance (6MWD) were compared among groups with different TSC2 status and genotypes to analyze genotype-phenotype correlations. The results showed 67 variants in 43 genes detected, with a TSC2 mutation detection rate of 68.2%. The TSC2 detection rate was similar in specimens obtained either through transbronchial lung biopsy (TBLB) or surgical lung biopsy A novel mutation in VEZF1 (c.A659G) was detected in four participants and may represent a mild disease state. TSC2 mutation was significantly related to a shorter six minute walk distance and a higher percentage of VEGF-D over 800 pg/mL and stop-gain mutation was significantly related to a higher prevalence of pneumothorax. The authors concluded that TSC2 mutation detectability and types are related to the disease severity and phenotypes of S-LAM.

Tian et al. (2021) conducted a study to examine the association between TSC mutation subtypes and the prevalence of LAM in women with TSC. 55 adult women seen at the Cincinnati Children's Hospital Medical Center's TSC clinic were stratified into the following three groups: those with TSC1 mutation, those with TSC2 mutation, and those with no mutation identified (NMI). TSC manifestations were ascertained by blinded review of chest computed tomographic scans (LAM, multifocal micronodular pneumocyte hyperplasia, and sclerotic bone lesions) and chart review (all other manifestations). The results showed that twenty-three women had characteristic cysts consistent with LAM, 16 of which had TSC2 mutations. The prevalence of LAM was higher in women with TSC2 mutations compared with women with TSC1 mutations. Renal angiomyolipomas were more common in women with TSC2 mutations compared with women with TSC1 mutations, and there was no association between TSC mutation subtype and the presence of multifocal micronodular pneumocyte hyperplasia, sclerotic bone lesions, and skin or brain involvement. Serum VEGF-D (vascular endothelial growth factor-D) concentrations tended to be higher in patients harboring TSC2 mutations compared with patients with TSC1 mutations and in patients with LAM compared with patients without LAM. The authors concluded that LAM and angiomyolipomas are more common in women with TSC harboring TSC2 mutations compared with women with TSC1 mutations.

FGFR1

Through a systematic review and meta-analysis, Hu et al. (2021) aimed to clarify the correlation between FGFR1 and the survival outcomes for individuals with head and neck squamous cell carcinoma (HNSCC). The results indicated that FGFR1 predicted poor overall survival (OS) in individuals with HNSCC. Furthermore, FGFR1 was related to poor OS for individuals with human papillomavirus (HPV) negative HNSCC, not in HPV-positive HNSCC. Subgroup analysis stratified by molecular abnormalities, such as overexpression or amplification, showed similar results. Limitations included: First, the numbers of articles used for assessing the association between FGFR1 and the prognosis of HNSCC were limited in the present meta-analysis, studies containing languages other than English, and the inclusion of unpublished data may contribute to additional bias, the results may be heterogeneous due to the utilization of different methods and statistical analysis. The authors concluded that the present study demonstrated that individuals with HNSCC and FGFR1 overexpression and amplification were more likely to exhibit poorer survival.

In a 2024 exploratory analysis of AGO-OVAR11/ICON-7, Heublein et al. (2024) sought to understand to what extent people benefit from antiangiogenic treatment with bevacizumab, either in combination with poly-(ADP-ribose) polymerase inhibitor or as a single-agent maintenance. As fibroblast growth factor receptors and their ligands (FGFRs/FGFs) are critical players in angiogenic signaling and have been linked to resistance to several drugs, we investigated the prognostic or predictive potential of FGFs/FGFRs signaling in the context of bevacizumab treatment within the prospective phase III AGO-OVAR11/ICON-7 study. FGFR1, FGFR2, FGFR3, FGFR4, FGF1, and FGF19 gene expressions were determined in 380 ovarian carcinoma tumor samples collected from German centers in the multicenter phase III AGO-OVAR11 trial/ICON-7 trial. All patients received carboplatin and paclitaxel, administered every three weeks for six cycles, and were randomized to bevacizumab. Expressions of FGFR1, FGFR2, FGF1, and FGF19 were associated with progression-free survival in both univariate and multivariate analysis. A signature built by FGFR1, FGFR4, and FGF19 defined a subgroup (n = 62) of patients that derived the most remarkable bevacizumab-associated improvement of progression-free survival (HR, 0.3; P = .004). In this exploratory analysis of a prospective randomized phase III trial, we provide evidence that the expression of FGFRs/ FGFs might have independent prognostic values. An FGFR/FGF-based gene signature identified in the study predicts long-term benefit from bevacizumab. This observation is hypothesis-generating and requires validation on independent cohorts.

In 2020, authors Abou-Alfa et al. conducted a multicenter, open-labeled, single-armed, multi-cohort, phase 2 study (FIGHT-202) on individuals 18 years or older to evaluate the safety and antitumor activity of pemigatinib for those previously treated, locally advanced or metastatic cholangiocarcinoma with and without FGFR2 fusions or rearrangements. All enrolled individuals received a starting dose of 13·5 mg oral pemigatinib one time a day (21-day cycle; 2 weeks on, one week off) until disease progression, unacceptable toxicity, withdrawal of consent, or physician decision. The outcome measured was the proportion of participants who achieved an objective response among those with FGFR2 fusions or rearrangements, assessed centrally in all those who received at least one dose of pemigatinib. Results of the study showed that overall, hyperphosphatasemia was the most common all-grade adverse event irrespective of cause (88 [60%] of 146 people). 93 (64%) participants had a grade 3 or worse adverse event (irrespective of cause); the most frequent were hypophosphatemia (18 [12%]), arthralgia (nine [6%]), stomatitis (eight [5%]), hyponatremia (eight [5%]), abdominal pain (seven [5%]), and fatigue (seven [5%]). 65 (45%) people had severe adverse events; the most frequent were abdominal pain (seven [5%]), pyrexia (seven [5%]), cholangitis (five [3%]), and pleural effusion (five [3%]). Overall, 71 (49%) people died during the study, most frequently because of disease progression (61 [42%]); no deaths were deemed to be treatment related. The authors concluded that this data supports the therapeutic capability of pemigatinib in formerly treated people with cholangiocarcinoma who have FGFR2 fusions or rearrangements.

In a 2020 human study of futibatinib (a fibroblast growth factor receptor [FGFR]) for individuals with advanced solid tumors, Bahleda et al. evaluated the safety and pharmacokinetics/pharmacodynamics (PK/PD) of futibatinib in advanced solid tumors. The results of the study showed that with a total of 86 participants enrolled in the nine t.i.w. (n = 42) and five q.d. cohorts (n = 44); 71 (83%) had tumors harboring FGF/FGFR aberrations. Three of nine people in the 24-mg q.d. cohort experienced dose-limiting toxicities, including grade 3 increases in alanine transaminase, aspartate transaminase, and blood bilirubin (n = 1 each). The maximum tolerated dose (MTD) was determined to be 20 mg q.d.; no MTD was defined for the t.i.w. schedule. Across cohorts (n = 86), the most common treatment-emergent adverse events (TEAEs) were hyperphosphatemia (59%), diarrhea (37%), and constipation (34%); 48% experienced grade 3 TEAEs. TEAEs led to dose interruptions, dose reductions, and treatment discontinuations in 55%, 14%, and 3% of people, respectively. Pharmacokinetics were dose proportional across all q.d. doses but not all t.i.w. doses evaluated, with saturation observed between 80 and 200 mg t.i.w. Serum phosphorus increased dose-dependently with futibatinib on both schedules, but a stronger exposure-response relationship was observed with q.d. dosing, supporting 20 mg q.d. as the recommended phase II dose (RP2D). Overall, partial responses were observed in five participants [FGFR2 fusion-positive intrahepatic cholangiocarcinoma (n = 3) and FGFR1-mutant primary brain tumor (n = 2)], and stable disease in 41 (48%). The authors concluded that this phase I dose-escalation trial demonstrated tolerability, PD activity, and preliminary antitumor activity of futibatinib in heavily pretreated patients with advanced solid tumors. Safety and PK data supported 20 mg futibatinib q.d. as the RP2D. Based on these results, futibatinib has been evaluated in the phase I dose-expansion portion of this study in multiple tumor types (recently completed) and in phase II registrational trial (NCT02052778) in iCCA with FGFR2 fusions/rearrangements. The phase II trial has completed recruitment, and results of interim analyses are expected in 2020. In addition, recruitment is ongoing in other phase II [NCT04024436 (breast cancer)] and phase III [NCT04093362 (iCCA)] trials.

MTOR

Moslehian et al. (2023) conducted a systematic review aimed to assess the possible influence of abnormal mTOR-associated long non-coding RNAs (lncRNAs) in the tumorigenesis of colorectal tissue. Twenty-four studies were assessed that were published from 2018 to 2022. The data showed that mTOR and downstream cascade can be stimulated and/or inhibited via different types of lncRNAs in colorectal cancer (CRC), indicating the existence of an intricate interaction between the mTOR signaling pathway and lncRNAs. The authors speculate from their conclusion that mTOR, and mTOR-associated pathways are among the potential factors affected by lncRNAs in most cancers. In addition, determining the dynamic activity of mTOR and relevant signaling pathways via lncRNAs can help progress novel molecular therapeutics and medications. The limitations of the study include small sample size and limited studies with insufficient data.

Lee et al. (2022) performed a systematic review and meta-analysis aimed to elucidate at the gene level the biological mechanisms involved in gastric cancer (GC) development and to identify candidate drug target genes. The meta-analysis identified 226 single nucleotide polymorphisms (SNPs) located in 91 genes associated with GC. Twelve studies published between 2008 and 2020 from multiple countries were utilized. The results of the analysis identified 44 significant genes for GC susceptibility. Among the 44 genes, 12 genes (THBS3, GBAP1, KRTCAP2, TRIM46, HCN3, MUC1, DAP3, EFNA1, MTX1, PRKAA1, PSCA, and ABO) were significant at the gene expression level. Using disease network and pathway analyses, the study identified that PRKAA1, THBS3, and EFNA1 were significantly associated with the PI3K-Alt-mTOR-signaling pathway, and that MUC1 acts as a regulator in both the PI3K-Alt-mTOR and P53 signaling pathways. The authors concluded that PRKAA1 involved in the PI3K-Alt-mTOR-signaling pathway, is a key gene for GC development and could be a target gene for drug development in the future. The limitations of the study include potential for publication bias, lack of follow-up analysis, possible bias related to heterogeneity in population origin, phenotype definition, genotyping platform and software.

Moura et al. (2021) conducted a systematic review and meta-analysis to determine the prevalence of PI3K-AKT-MTOR signaling pathway mutations in patients with head and neck cancer (HNC). The 105 studies included in the quantitative synthesis of the mutational prevalence comprised a total of 8360 samples with 1306 mutations. In the pooled data, 20 articles investigated MTOR mutations. Of the 1658 samples tested a total of 38 MTOR mutations were reported among patients with HNC. The overall pooled prevalence of MTOR mutations in HNC was 3% (95 % CI = 2–4%; p = 0.40; I^2 = 5%). Missense mutations were more frequent and found in 79 % of samples. The authors concluded that the PI3K-AKT-mTOR pathway is a potentially promising biomarker in HNC and offers a molecular basis for future personalized targeting therapeutic studies. The limitations of the study include potential selection bias, research design, and study methodology.

BIRC3

Diop et al. (2020) analyzed, in a retrospective cohort study, the prognostic impact of BIRC3 mutations in 287 individuals receiving first-line fludarabine, cyclophosphamide, and rituximab (FCR). BIRC3 is a recurrently mutated gene in patients with chronic lymphocytic leukemia (CLL) and disease with this mutation is less sensitive to fludarabine-based chemoimmunotherapy. BIRC3 mutations were analyzed by targeted next-generation sequencing of 24 recurrently

mutated genes in CLL. The results showed BIRC3 mutations identify a poor prognostic subgroup of patients in whom FCR treatment fails. BIRC3 mutations may be a molecular predictor of FCR resistance that could be used to select patients to be treated with novel targeted agents.

Blakemore et al. (2020) studied randomized patient samples of 499 patients diagnosed with CLL targeted resequencing of 22 genes and identified 623 mutations which included biallelic BIRC3 lesions (mutation and deletion) as an independent marker of inferior progression free survival (PFS) and overall survival (OS). The results showed that cases with a biallelic BIRC3 had a significantly reduced OS, suggesting that biallelic loss of BIRC3 represents the subgroup of 11q deleted CLL with the worst outcome following initial treatment with chemotherapy and were found to be independent prognostic markers for PFS and OS. The authors concluded that these findings show that a more expansive genomic screening approach provides additional clinical information, thereby helping to establish the precise importance of genetic alterations in the context of other established and emerging biomarkers.

FBXW7

Else et al. (2021) conducted a Randomized Controlled Trial to investigate the cause of death, in particular, deaths due to infection in chronic lymphocytic leukemia with long-term follow-up. The authors examined the causes of death for 600 individuals in the LRF CLL (chronic lymphocytic leukemia) 4 trial and randomly took blood samples from 499 individuals to identify gene mutations. The results of the trial showed that Infection was a cause of death in 258 people (43%). Individuals dying of infection were more likely than those who died of other origins to have received ≥ 2 lines of treatment (194/258 [75%] versus 231/342 [68%]) and to have died in the winter months (149/258 [58%] versus 166/342 [49%]), respectively. In those with mutation data, the circumstances significantly linked with death from infection versus all other deaths were 11q deletion (47/162 [29%] versus 40/209 [19%]) and mutations of the BRAF, FBXW7, NRAS, and XPO1 genes. An infection caused death in 46/67 quantifiable individuals (69%) who had a mutation of one or more of these four genes versus 129/333 (39%) lacking any of these mutations. The main limitations of this study were the small number of people with each of these gene mutations; the conclusion of clinical follow-up in 2010, with the outcome that we have no data on the treatments received by these individuals since then; and the fact that the randomized treatments in LRF CLL4 are no longer in general use, raising the question as to whether deaths from infection may follow a dissimilar pattern in the era of B-cell receptor (BCR) and BCL2 inhibitors. The authors concluded that people in LRF CLL4 were at some risk of death from infection, regardless of their demographic characteristics, disease stage, and treatment history. However, those who had obtained two or more lines of treatment were markedly at risk, as were those who carried a BRAF, FBXW7, NRAS, or XPO1 mutation. A meta-analysis of datasets from other trials could be valuable in measuring the weight of the link between these gene mutations and deaths from infections for those with CLL. Cautious management of infection risk, involving proper choice of CLL therapeutic agents and prophylaxis against infection, may be valuable for those who carry one or more of these mutations.

In a systematic review and meta-analysis conducted by Shang et al. (2021), the authors sought to summarize the earlier evidence and evaluate the clinical significance, having the prognostic role, of FBXW7 status in CRCs (colorectal cancer). The results of the investigations showed that ten studies involving 4199 people met the inclusion criteria and were included in the meta-analysis. FBXW7 mutation/low expression was associated with advanced T stage and lymph node metastasis but was not related to other parameters. Further examination discovered that FBXW7 mutation/low expression predicted poor OS, but not disease-free survival (DFS) in CRC. The subgroup analysis uncovered that FBXW7 status was correlated with OS in cohorts enlisted after 2009 from eastern Asia, detected by immunohistochemistry/qRT-PCR, and examined with multivariate method. The limitations of this investigation were significant heterogeneity among the included studies, there was some inevitable variability in study designs, several studies had a small number of participants recruited, and publication bias. The authors concluded that altered FBXW7 status was correlated with advanced T stage and lymph node metastasis in CRC, and low FBXW7 mRNA/protein level shows poor OS in CRC. FBXW7 may be a prognostic biomarker for individuals with CRC. These conclusions may provide evidence for deciding a therapeutic regimen for people with CRC.

JAK1, JAK3

Wahnschaffe et al. (2019) performed a meta-analysis to evaluate the genomic aberrations affecting JAK/STAT signaling in T-cell prolymphocytic leukemia (T-PLL) and developed a model of mechanisms leading to JAK/STAT signaling in T-PLL cells. Most frequently, JAK1 (6.3%), JAK3 (36.4%), and STAT5B (18.8%) carried somatic single-nucleotide variants (SNVs), with missense mutations in the SH2 or pseudokinase domains as most prevalent. These lesions were predominantly subclonal. There was no strong association detected between mutations of a JAK or STAT gene with clinical characteristics. Irrespective of the presence of gain-of-function (GOF) SNVs, basal phosphorylation of STAT5B was elevated in all analyzed T-PLL. A significant proportion of genes encoding for potential negative regulators of STAT5B showed genomic losses (in 71.4% of T-PLL in total, in 68.4% of T-PLL without any JAK or STAT mutations). They included DUSP4, CD45, TCPTP, SHP1, SOCS1, SOCS3, and HDAC9. Overall, considering such losses of negative

regulators and the GOF mutations in JAK and STAT genes, a total of 89.8% of T-PLL revealed a genomic aberration potentially explaining enhanced STAT5B activity. According to the authors, this review presents prevalent genomic lesions that affect genes encoding JAK/STAT signaling components and provides an overview of possible modes of activation of this pathway in a cohort of T-PLL. Additionally, this review supplies a framework for strategies to inhibit JAK/STAT signaling in T-PLL, even in individuals whose leukemia does not carry mutations in a JAK or STAT gene.

Stengel et al. (2016) performed a cytogenic and molecular genetic characterization of 51 individuals (33 male and 18 female) with newly diagnosed T-PLL, including potential associations between respective markers and their impact on prognosis. Genetic abnormalities were found in all the 51 cases with T-PLL, most frequently involving the TCRA/D locus (86%). Deletions were detected for ATM (69%) and TP53 (31%), whereas i(8)(q10) was observed in 61% of cases. Mutations in ATM, TP53, JAK1, and JAK3 were detected in 73, 14, 6, and 21% of patients, respectively. Additionally, BCOR mutations were observed for the first time in a lymphoid malignancy (8%). Two distinct genetic subgroups of T-PLL were identified: A large subset (86% of patients) showed abnormalities involving the TCRA/D locus activating the proto-oncogenes TCL1 or MTCP1, while the second group was characterized by a high frequency of TP53 mutations (4/7 cases). According to the authors, JAK3 mutations function as important prognostic marker, showing a significant negative impact on overall survival (11 months vs. 37 months) which suggests that those with T-PLL could benefit from inhibitors targeting the JAK-STAT pathway, particularly JAK3 inhibitors.

Bergamann et al. (2014) analyzed a cohort of 32 T-PLL individuals for mutations in the JAK3 gene and 14 mutations in 11 of 32 individuals (34%) were identified. M5I II (seen in 57% of cases) previously described as an activating change in other T-cell malignancies was the most frequently detected mutation in the cohort. Three patients carried two mutations in JAK3. In two patients M5I II and R657Q were simultaneously detected and in another patient V674F and V678L. The latter case demonstrated that the mutations were on the same allele in cis. Protein modeling and homology analyses of mutations present in other members of the JAK family suggested that these mutations likely activate JAK3, possibly by disrupting the activation loop and the interface between N and C lobes, increasing the accessibility of the catalytic loop. In addition, four of the 21 patients lacking a JAK3 point mutation presented an aberrant karyotype involving the chromosomal band 19p13 harboring the JAK3 locus. The authors concluded that recurrent activating JAK3 mutations in those with T-PLL could enable the use of JAK3 inhibitors in the treatment of this aggressive malignancy. Limitations include small study size.

STAT5B

In a 2024 review, Liongue et al. presented new insight on the role of STAT proteins and gene mutations on myeloproliferative neoplasms (MPNs) to present a unifying molecular etiology in which these overlapping diseases are best understood. Specifically, the chronic activation of signaling pathways, most notably STAT5B, leading to the sustained stimulation of myelopoiesis, which underpins the various disease sequalae. Multiple gene mutations are associated with MPNs, and somatic GOF mutations of the STAT5B gene have been demonstrated in patients diagnosed with hypereosinophilic syndrome and CNL. Despite the heterogeneity in the underlying mutations, there is overwhelming evidence that chronic STAT activation, most notably STAT5B, is central to mediating the impacts of the various mutations at the cellular level The central role of chronic STAT activation in MPNs, particularly of STAT5, makes the restoration of signaling to non-pathological levels an attractive goal for therapeutic intervention. Pharmacological agents that have displayed efficacy against relevant MPN-associated mutants have almost uniformly demonstrated an associated significant reduction in STAT5 activation. A range of these are in various stages of development, including those targeting activation, DNA binding, dimerization, or translation. This understanding of the central role played by chronic STAT5 activation provides a view into the various clinical manifestations of MPNs, including the overlapping nature of different categories of MPNs, and, importantly, the approaches that may provide therapeutic benefit.

Smith et al. (2023) conducted a review to addresses the biology of STAT5 proteins and discuss STAT5's role within the context of human disease. In humans, the identification of deleterious STAT5B mutations demonstrates that STAT5a is unable to compensate for the absence of STAT5b, despite their high amino acid similarity. Pathologic mutations affecting its function and signaling have been described in all domains of STAT5b. Thus far, different molecular mechanisms of STAT5B mutations have been identified. Autosomal recessive loss-of-function (LOF) mutations and heterozygous dominant negative mutations as well as recently, somatic gain-of-function (GOF) mutations, associated with severe allergic inflammation and hematological malignancies, have been reported.

Pathfinder TG®/PancraGEN

Loren and colleagues (2016) retrospectively studied whether initial adjunctive IMP testing using the PancraGEN test affected future real-world pancreatic cyst management decisions for intervention or surveillance relative to the ICG recommendations, and if this resulted in improved individual outcomes. This review used data from the National Pancreatic Cyst Registry. Participants in this registry had received IMP testing at the discretion of their treating physician.

Details of how that decision was made are not available. Researchers evaluated the relationship between real-world decisions (intervention vs. surveillance), ICG model recommendations (surgery vs. surveillance) and IMP (PancraGEN) diagnoses (high-risk vs. low-risk). Kaplan Meier and hazard ratio analyses as well as 2 × 2 tables were used to assess time to malignancy. Logistic regression was used to determine odds ratios for surgery decision. Of 491 patients, 206 received clinical intervention at follow-up (183 surgery, 4 chemotherapy, 19 presumed by malignant cytology). Overall, 13 % (66/491) of patients had a malignant outcome and 87 % (425/491) had a benign outcome at 2.9 years' follow-up. When ICG and IMP were concordant for surveillance/surgery recommendations, 83 % and 88 % actually underwent surveillance or surgery, respectively. However, when discordant, IMP diagnoses were predictive of real-world decisions, with 88 % of patients having an intervention when ICG recommended surveillance, but IMP indicated high risk, and 55 % undergoing surveillance when ICG recommended surgery, but IMP indicated low risk. These IMP-associated management decisions benefitted patient outcomes in these subgroups, as 57 % had malignant and 99 % had benign outcomes at a median 2.9 years' follow-up. IMP was also more predictive of real-world decisions than ICG by multivariate analysis: OR 11.4 (95 % CI 6.0 − 23.7) versus 3.7 (2.4 − 5.8), respectively. A study limitation is that the outcomes data were obtained by review of medical records from individuals previously tested by IMP. The study is also limited by potential inclusion bias due to the lack of information about how the decision to use IMP testing was made. The retrospective design limits generalizability to prospective decision making. Additional well-designed studies are needed.

BCR-ABL

Arber et al. (2022) presented the International Consensus Classification (ICC) of myeloid and lymphoid neoplasms classification to integrate genomic information to allow therapy to be more easily tailored to targeted agents. The authors propose that the ICC be adapted to an interactive web-based portal to allow more nimble modification to reflect new discoveries and improve diagnostic, prognostic, and predictive information. The updated classification includes some revisions from the 2016 WHO classification and introduces a number of new subtypes. BCR::ABL1-positive ALL from the prior classification is now divided into 2 biologically distinct subsets that cannot be distinguished by the use of p190 vs p210 fusion proteins, but rather by fluorescence in situ hybridization (FISH), based on whether the translocation can be detected in granulocytes, indicating a multilineage BCR::ABL1 fusion. Further evidence comes from the finding that RT-PCR studies for BCR::ABL1 following treatment may show high level positivity when both flow cytometry and molecular MRD methods show no or little evidence of MRD. Prognosis and optimal treatment of these 2 variants may also differ. Cases of B-ALL, BCR::ABL1 like are now recognized as having a wide variety of genetic lesions including JAK-STAT alterations.

EGFR/ InVisionFirst, Liquid Biopsy

Plagnol et al. (2018) developed the InVisionFirst™ assay, an assay based on enhanced tagged amplicon sequencing (eTAm-Seq™) technology to profile 36 genes commonly mutated in non-small cell lung cancer (NSCLC) and other cancer types for actionable genomic alterations in cell-free DNA. The assay has been developed to detect point mutations, indels, amplifications and gene fusions that commonly occur in NSCLC. Results were compared with digital PCR (dPCR). The InVisionFirst assay demonstrated an excellent limit of detection, with 99.48% sensitivity for SNVs present at VAF range 0.25%-0.33%, 92.46% sensitivity for indels at 0.25% VAF and a high rate of detection at lower frequencies while retaining high specificity (99.9997% per base). The assay also detected ALK and ROS1 gene fusions, and DNA amplifications in ERBB2, FGFR1, MET and EGFR with high sensitivity and specificity. Comparison between the InVisionFirst assay and dPCR in a series of cancer patients showed high concordance. This analytical validation demonstrated that the InVisionFirst assay is highly sensitive, specific and robust, and meets analytical requirements for clinical applications. This study demonstrates that the InVisionFirst assay has high analytical sensitivity, specificity and reproducibility which are appropriate for clinical applications. Separate studies are ongoing to test clinical validity and utility in a range of settings.

Yi et al. (2024) reported the analytical and clinical validation of a next-generation sequencing (NGS)–based circulating tumor DNA (ctDNA) assay in Non–Small Cell Lung Cancer (NSCLC) by evaluating the limit of detection (LOD), precision, and specificity for various genomic aberrations. The real-world performance in NSCLC was assessed by comparing the results of AlphaLiquid100 to the tissue-based results. The LODs with 30 ng input DNA were 0.11%, 0.11%, 0.06%, 0.21%, and 2.13 copies for detecting single nucleotide variants, insertions, deletions, fusions, and copy number alterations (CNA), respectively. Quantitatively, single nucleotide variants/insertions and deletions, fusions, and CNAs showed a good correlation to the manufacturer's values, and per-base specificities for all types of variants were near 100%. In real-world NSCLC (n = 122), key actionable mutations in NSCLC were detected in 60.7% (74/122) with the ctDNA assay. Comparative analysis against the NGS-based tissue results for all key mutations showed positive percent agreement (PPA) of 85.3%. For individual genes, the PPA was as high as 95.7% for epidermal growth factor receptor (EGFR) mutations and 83.3% for ALK translocations. AlphaLiquid100 detected drug-sensitive EGFR mutation at a variant allele frequency as low as 0.02% and also identified an EGFR mutation in a case where tissue sample missed. Blood samples collected post-targeted therapies revealed additional acquired mutations. The authors concluded that the

AlphaLiquid100 ctDNA assay demonstrates robust analytical validity, offering clinically important information for NSCLC patients.

Germline Testing for Use of PARP Inhibitors

LaDuca et al. (2020) systematically evaluated 32 cancer predisposition genes by assessing phenotype-specific pathogenic variant (PV) frequencies, cancer risk associations, and performance of genetic testing criteria in a cohort of 165,000 patients referred for multigene panel testing (MGPT). Patients underwent comprehensive germline analysis of 5–49 genes depending on the multigene panel ordered. With the exception of GREM1 and EPCAM, either Sanger or next-generation sequencing analysis was performed for all coding domains. The study identified genetic heterogeneity surrounding predisposition to cancer types commonly referred for germline testing, such as breast, ovarian, colorectal, uterine/endometrial, pancreatic, and melanoma. PV frequencies were highest among patients with ovarian cancer (13.8%) and lowest among patients with melanoma (8.1%). Fewer than half of PVs identified in patients meeting testing criteria for only BRCA1/2 or only Lynch syndrome occurred in the respective genes (33.1% and 46.2%). In addition, 5.8% of patients with PVs in BRCA1/2 and 26.9% of patients with PVs in Lynch syndrome genes did not meet respective testing criteria. Limitations include risk estimates limited by size for cancers other than breast, and cancer risk analysis was not informative for many genes because of low PV count, and limitations exist surrounding the cohorts used for case-control analysis

Taylor et al. (2021) identified multiple randomized controlled trials on the safety, efficacy, and potential harms of poly (ADP-Ribose) polymerase (PARP) inhibitors in the treatment of patients with locally advanced or metastatic breast cancer. Overall survival was the primary outcome, and secondary outcomes were progression-free survival, tumor response rate, quality of life, and adverse events. The authors reported on those with locally advanced or metastatic HER2-negative (people with breast cancer that tests negative for a protein called human epidermal growth factor receptor 2), BRCA germline mutated (participant carries a mutation in the BRCA gene) breast cancer. Their findings on PARP inhibitors include: risk reduction of death by 13%, risk reduction of disease growth by 37%, chance of tumor shrinkage (66.9% for PARP inhibitors versus 48.9% for other treatments), and little to no difference in side effects compared to other treatment arms. Quality of life data were collected in two trials, and available evidence showed PARP inhibitors were superior compared to the physician's choice of chemotherapy for participant-reported outcomes. The most common adverse events reported across all studies included neutropenia, anemia, and fatigue. The authors concluded that PAPR inhibitors offer a progression-free survival advantage. Given the fact that PARP inhibitors had no significant increase in toxicity, they could be incorporated into the treatment paradigm for patients with locally advanced/metastatic HER2-negative, BRCA germline mutated breast cancer. A study limitation would be the availability of only three phase 3 clinical trials and the differing comparator arms of PARP inhibitor plus chemotherapy versus chemotherapy alone compared to single agent PARP inhibitor versus physician's choice of chemotherapy. Future studies with sufficient power to detect meaningful improvements in overall survival are warranted.

Guardant360®

Rozenblum et al. (2017) studied tissue biopsies from 101 advanced NSCLC patients were tested locally for EGFR mutations and ALK fusions. Tissue-based CGP identified 15 EGFR and ALK alterations missed locally, but could only be performed in 82 of the 101 (81%) patients because of tissue exhaustion. Guardant360® was used in the 19 remaining patients, and two (11%) additional sensitizing EGFR mutations were found that had been missed with local tissue genotyping. In addition, alterations including MET amplification, ERBB2 (HER2) mutation, and two RET fusions were also identified (missed with local non-CGP genotyping), for a total of 6 driver alterations in 19 patients (32%). Thus, Guardant360 changed treatment in 32% of patients with insufficient samples for tissue-based CGP, with five receiving matched therapy. These five patients achieved a 60% objective response rate and a 100% disease control rate.

Aggarwal et al. (2018) examined the clinical implications of using plasma-based testing in addition to tissue-based testing in 229 patients with NSCLC. Of the 229 patients in whom both tissue and plasma testing were ordered, the addition of plasma increased the percentage of patients eligible for targeted therapies from 21% (47/229) to 36% (82/229). For the 128 patients with successful tissue testing results, 55 were found to have a therapeutically targetable mutation. Of these 55, only 31 had this mutation found in tissue and plasma, though not necessarily the same actionable mutation(s) in each testing method. For 16 patients, the mutation was found in tissue only, and for 8, it was found in plasma only. To further assess whether the selection of targeted therapy based on the detection of low allele frequency mutations that Guardant360® is able to identify has a clinical benefit, the authors assessed the depth of response to targeted mutations identified in plasma-based testing. A total of 42 patients received a targeted therapy consistent with the plasma-based testing, 12 of whom had that mutation also detectable in tissue-based testing as well. Of this 42, there were 36 (85.7%) who achieved a response of stable disease, partial response, or complete response.

KRAS

Prior et al. (2020) cross-referenced data in all major publicly accessible cancer mutation databases to determine mutation frequency values for each Ras isoform in all major cancer types. It was concluded that the global disease burden associated with Ras mutations for different cancer types showed approximately 19% of patients with cancer harbor Ras mutations with KRAS responsible for 75% of that number. More research and larger databases with genome wide profiling will allow informed precision medicine for cancer treatments.

Ma et al. (2017) compared mutations detected in EGFR, KRAS, and BRAF genes using next-generation sequencing (NGS) and confirmed by Sanger sequencing with mutations that could be detected by FDA-cleared testing kits. Paraffin-embedded tissue from 822 patients was tested for mutations in EGFR, KRAS, and BRAF by NGS. Sanger sequencing of hot spots was used with locked nucleic acid to increase sensitivity for specific hot-spot mutations. This included 442 (54%) lung cancers, 168 (20%) colorectal cancers, 29 (4%) brain tumors, 33 (4%) melanomas, 14 (2%) thyroid cancers, and 16% others (pancreas, head and neck, and cancer of unknown origin). Results were compared with the approved list of detectable mutations in FDA kits for EGFR, KRAS, and BRAF. Of the 101 patients with EGFR abnormalities as detected by NGS, only 58 (57%) were detectable by cobas v2 and only 35 (35%) by therascreen. Therefore, 42 and 65%, respectively, more mutations were detected by NGS, including two patients with EGFR amplification. Of the 117 patients with BRAF mutation detected by NGS, 62 (53%) mutations were within codon 600, detectable by commercial kits, but 55 (47%) of the mutations were outside codon V600, detected by NGS only. Of the 321 patients with mutations in KRAS detected by NGS, 284 (88.5%) had mutations detectable by therascreen and 300 (93.5%) had mutations detectable by cobas. Therefore,11.5 and 6.5% additional KRAS mutations were detected by NGS, respectively. Conclusion NGS provides significantly more comprehensive testing for mutations as compared with FDA-cleared kits currently available commercially.

MammaPrint®

In 2022, Vliek and colleagues published a ten-year follow-up of the observational RASTER study. The prospective RASTER study assessed the tumors of 427 individuals with cTanyN0M0 breast cancer. The study aimed to decide the 70-gene signature (MammaPrint)'s ability to guide adjuvant chemotherapy decisions for individuals with estrogen receptor (ER) + and human epidermal growth factor receptor 2 (HER2)- breast cancer. The authors evaluated 310 of the 427 individuals at ten years of follow-up. Of the clinically high-risk individuals, 45 (49%) were classified as genomically low risk. In this subcategory, at ten years, distant recurrence-free interval (DRFI) was comparable among individuals treated with (95.7% [95% CI 87.7–100]) and without (95.5% [95% CI 87.1–100]) chemotherapy. In the group of clinically low-risk individuals, 56 (26%) were classified as genomically high-risk. For the clinically low-risk group, a variance was seen among the genomically high- and low-risk subgroup after five years, resulting in a 10-year DRFI of 84.3% (95% CI 74.8–95.0) and 93.4% (95% CI 89.5–97.5), respectively. Genomic ultralow-risk individuals' outcomes were a 10-year DRFI of 96.7% (95% CI 90.5–100), primarily (79%) without systemic therapy. Limitations to the RASTER study include the observational nature and the risk of bias. The authors concluded that over ten years, individuals with clinically high-risk, genomic low-risk tumors have excellent results irrespective of the use of chemotherapy. The updated outcomes of the MINDACT trial and RASTER study collectively demonstrate that the data supports the use of the MammaPrint, in ER +, HER2-, and N0, clinically high-risk individuals with breast cancer.

In 2021, Piccart et al. produced updated results on phase 3 randomized MINDACT trial, including long-term follow-up with an exploratory analysis by age. MINDACT was a randomized, phase 3, multicenter trial conducted in 112 academic and community hospitals in nine countries that enrolled individuals that had confirmed primary invasive breast cancer with N1, no distant metastases, and a WHO performance status of 0-1, and their genomic risk was decided using the MammaPrint 70-gene signature. Enrolled in the trial were 6,693 individuals with a mean follow-up of 8.7 years. The 8-year estimates for DMFS in the intention-to-treat population were 92.0% for chemotherapy set against 89.4% who received no chemotherapy. The 8-year DMFS in the exploratory analysis by nodal status in these individuals was 91.7% with chemotherapy and 89.2% without chemotherapy in 699 N0 individuals (absolute difference 2.5 percentage points and 91.2% as opposed to 89.9% for 658 individuals with N1). The exploratory analysis conducted to determine the effects of chemotherapy administration on 8-year DMFS according to age resulted in 93.6% with chemotherapy set against 88.6% without chemotherapy in 464 women aged 50 years or younger and 90.2% vs. 90.0% in 894 women older than 50 years. This long-term follow-up of phase 3 randomized MINDACT trial showed the 70-gene signature's capability of detecting women with high clinical risk, a subgroup, and specific individuals with low genomic risk, with an exceptional DMFS when treated with endocrine therapy by itself. For this group of women, the size of the profit from adding chemotherapy to endocrine therapy continues to be small and is not improved by nodal positivity. The benefit is age-dependent and is solely seen in women under 50; further study is needed in younger women, who may need reinforced endocrine therapy to forego chemotherapy. The authors concluded that MammaPrint ought to be a portion of informed, shared decision-making.

Melanoma Risk Stratification Molecular Testing

Gerami et al. (2015) identified a distinct gene expression profile (GEP) signature that characterizes high-risk and low-risk subtypes of cutaneous melanoma that is an independent prognostic marker in multivariate analysis when analyzed alongside traditional melanoma staging defined by the American Joint Committee on Cancer (AJCC). GEP signatures have been shown to have powerful prognostic capabilities for many tumors and provide a significant improvement in prognostic accuracy compared with classification by TNM staging criteria. The study combines quantitative genetic expression analysis of a large, multicenter cohort of primary formalin-fixed, paraffin-embedded (FFPE) melanoma cases with the development of a clinically validated prognostic training set. Kaplan–Meier analysis indicated that the 5-year disease-free survival (DFS) rates in the development set were 100% and 38% for predicted classes 1 and 2 cases, respectively. DFS rates for the validation set were 97% and 31% for predicted classes 1 and 2 cases, respectively. The authors concluded that the prognostic power of this assay is considerably greater than other reported prognostic assays for melanoma. The identification of stage I and II SLN-negative patients at high risk for recurrence allows these patients to take part in more aggressive imaging protocols for early detection of metastatic disease and to be considered for adjuvant therapy.

Vetto et al. (2019) conducted a study to assess if gene expression profiling (GEP) can be used to identify patients with stage T1–T2 melanoma at low risk for sentinel lymph node (SLN) positivity. Patients with a positive SLN are at substantially increased risk for distant metastatic disease and death. The Multicenter Selective Lymphadenectomy Trial (MSLT-I) reported that the SLNB procedure provides prognostic information but does not appear to improve melanoma-specific survival (MSS). Bioinformatics modeling determined a population in which a 31-gene expression profile test predicted < 5% SLN positivity. Multicenter, prospectively-tested (n = 1421) and retrospective (n = 690) cohorts were used for validation and outcomes. The authors reported that patients 55–64 years with a class 1A (low-risk) profile had an SLN positivity rates of 4.9%. The Medicare population (≥ 65) with a class 1A, T1–T2 tumor had an even lower rate of SLN positivity at 1.6%, which is well within the NCCN “Do Not Recommend” category. Class 2B (high-risk) patients had SLN positivity rates of 30.8% and 11.9%. Melanoma-specific survival was 99.3% for patients ≥ 55 years with class 1A, T1–T2 tumors and 55.0% for class 2B, SLN-positive, T1–T2 tumors. The authors concluded that the 31-gene expression profile test identifies patients who could potentially avoid SLN biopsy, and it could be helpful in making decisions about the SLNB in a population with a higher frequency of comorbidities and for which the procedure already shows a lower yield. The study limitations include no long-term follow up is available for patients in the prospective cohorts, but the retrospective cohorts have long-term outcomes. A second multi-center validation study is ongoing to evaluate patients with T1–T2 melanoma who were clinically tested with the 31-GEP test and their SLNB results.

Greenhaw et al. (2020) conducted a meta-analysis to determine the overall effect of a 31-gene expression profile prognostic test in 1,479 patients. Clinical outcome metrics for this GEP was compared with the American Joint Committee on Cancer (AJCC) staging. The authors reported that the test result can sub-stratify AJCC-staged patients to increase or decreased risk of recurrence or distant metastasis, augmenting staging by the committee alone, which his consistent with previous reports. It reclassifies patients for heightened surveillance who were previously designated as being at low risk. Five-year recurrence-free and distant metastasis-free survival rates were 91.4% and 94.1% for Class 1A patients and 43.6% and 55.5% for Class 2B patients. The authors concluded that when the 31-gene expression profile and sentinel lymph node biopsy results were considered together, sensitivity and negative predictive value for distant metastasis-free survival were both improved. A limitation of this meta-analysis is that studies identified through systematic review are published, and unpublished negative-result data were not considered. Further studies are needed to evaluate appropriate methods and intervals for follow-up of high risk patients identified by the 31-gene expression profile, as well as therapeutic management, based on risk determined by the 31-GEP test together with other clinicopathologic covariates.

Molecular Assays for the Diagnosis of Cutaneous Melanoma

Clarke et al. (2020) conducted a retrospective study evaluating the accuracy of a 23-gene expression signature in differentiating melanoma and benign nevi. One hundred and eighty-one lesions were examined by seven dermatopathologists blinded to gene expression test results and clinical outcomes to identify diagnostically uncertain neoplasms. Of the 181 cases, 83 had benign clinical outcomes and 98 had malignant outcomes. An average age of 63 was reported for those with malignant and 42 years of age for those with benign outcomes. Out of the malignant lesions, 67% were from males and 33% were from females. Median follow-up time for benign lesions was 74.9 months, and 69% of cases had follow-up of at least 5 years (60 months). Median time to metastasis for the malignant cases was 17 months. There were 125 cases that met predetermined criteria for diagnostic uncertainty; this target population had a 90.4% sensitivity and 95.5% negative agreement of the test. The study showed high accuracy of the 23-gene expression signature within diagnostically uncertain lesions.

Estrada et al. (2020) assessed the development and validation of a diagnostic 35-gene expression profile test for ambiguous or difficult to diagnose suspicious pigmented skin lesions. A total of 951 samples (498 benign and 453

malignant) were evaluated via Quantitative RT-PCR in a multicenter cohort. The majority of malignant lesions were biopsied from arms and legs, while benign lesions were mainly located on patients' backs. The 35-GEP test to distinguish benign from malignant pigmented lesions was developed to improve diagnostic accuracy and reduce diagnostic uncertainty for difficult-to-diagnose cases. Unlike FISH, CGH or IHC, gene expression profiling captures transcriptomic events within the lesion and the surrounding tissue, allowing for a more comprehensive assessment of the biological changes that are associated with the transition to a malignant phenotype. IHC generally allows for evaluation of changes in the expression of a single biomarker at the protein level, which can be limited by subjective quantification systems. PRAME IHC has been reported as a reliable method to distinguish benign from malignant pigmented lesions; however, ~14% of nevi can have some staining for PRAME, and the interpretation of positive staining can be subjective. Thus, PRAME IHC requires further validation for widespread clinical use due to the potential for misdiagnosis of benign lesions as malignant. In the current study, PRAME expression did not improve diagnostic accuracy above the results reported for the 35-GEP.

Lezcano et al. (2020) analyzed 110 diagnostically problematic melanocytic tumors comparing results for (PReferentially expressed Antigen in MElanoma) PRAME immunohistochemistry (IHC) with those from fluorescence in situ hybridization (FISH) and/or single nucleotide polymorphism (SNP)-array, and each with the final diagnostic interpretation. The authors reported that in 90% of cases, there was concordance between PRAME IHC and cytogenetic tests results, and in 92.7% concordance between PRAME IHC and the final diagnosis. The high concordance between PRAME IHC and cytogenetic test results and the final diagnosis supports the use of PRAME IHC as an ancillary test in the evaluation of ambiguous primary cutaneous melanocytic neoplasms, especially with its faster turnaround over cytogenetic or gene expression studies. However, the authors found that the results indicate that PRAME IHC and cytogenetic tests for melanocytic tumors are not entirely interchangeable and occasionally each test type may yield false-negative or false-positive results. Limitations of this study include a low number of cases with a final diagnosis of melanoma (28 out of 110) but reflects the composition of most cohorts of ambiguous tumors. Further studies with longer follow up are necessary to assess further the sensitivity and specificity of PRAME IHC in ambiguous melanocytic tumors.

Microsatellite Instability-High (MSI-H) and Mismatch Repair Deficient (dMMR) Biomarker for Patients With Unresectable or Metastatic Solid Tumors

Marabelle et al. (2020) reported data from a nonrandomized, open-label, multi-site phase II KEYNOTE-158 multicohort study that enrolled patients with various noncolorectal high microsatellite instability/mismatch repair–deficient cancer who experienced failure with prior therapy with a median age of 60 years. Eligible patients received pembrolizumab 200 mg once every 3 weeks for 2 years or until disease progression, unacceptable toxicity, or patient withdrawal. Radiologic imaging was performed every 9 weeks for the first year of therapy and every 12 weeks thereafter. Among 233 enrolled patients, 27 tumor types were represented, with endometrial, gastric, cholangiocarcinoma, and pancreatic cancers being the most common. Median follow up was 13.4 months. Objective response rate was 34.3%. Median progression-free survival was 4.1 months, and median overall survival was 23.5 months. Treatment-related adverse events occurred in 151 patients (64.8%). Thirty-four patients (14.6%) had grade 3 to 5 treatment-related adverse events. Grade 5 pneumonia occurred in one patient; there were no other treatment related fatal adverse events. Because of the biologic role of MSI-H/dMMR in tumor pathophysiology, there has been great interest in the use of the MSI-H/dMMR biomarker as a potential predictor of response to pembrolizumab treatment based on a common tumor biomarker rather than on the anatomic location of origin. MMR/MSI status was evaluated by either immunohistochemistry assessing four MMR enzymes or PCR-based assessment of five microsatellite loci optimized to detect MSI in colorectal cancer. The latter approach may fail to detect some noncolorectal cancers since these five microsatellites may be less relevant in other tumor types. Molecular diagnostic tests that evaluate scores of microsatellites using next-generation sequencing will be important to evaluate the prevalence of MSI-H in noncolorectal cancers.

Ratovomanana et al. (2021) study aimed to evaluate and improve the performance of next-generation sequencing (NGS) to identify microsatellite instability (MSI) arising from defective mismatch repair (dMMR) in patients with metastatic colorectal cancer (mCRC) before treatment with immune checkpoint inhibitors (ICI). In this post hoc study, CRC samples were reassessed centrally for MSI and dMMR status using the reference methods of pentaplex polymerase chain reaction (PCR) and immunohistochemistry (IHC). Whole-exome sequencing (WES) was used to evaluate MSISensor, which is an FDA-approved and NGS-based method for assessment of MSI. This was performed in a prospective, multicenter cohort of 102 patients with mCRC from 2 clinical trials (C1); an independent retrospective, multicenter cohort of 113 patients (dMMR/MSI untreated with ICI) (C2); and a publicly available series of 118 patients with CRC from The Cancer Genome Atlas (C3). A new NGS-based algorithm (MSICare) was developed. Its performance for assessment of MSI was compared with MSISensor at the exome level or after down-sampling sequencing data to the MSK-IMPACT gene panel. MSICare was validated in an additional retrospective, multicenter cohort of 152 patients with new CRC enriched in tumors deficient in MSH6 and PMS2 after targeted sequencing of samples with an optimized set of microsatellite markers (MSIDIAG) (C4). The authors reported that at the exome level, MSISensor was highly specific but failed to diagnose MSI in 16% of MSI/dMMR mCRC from C1, 32% of mCRC, and 9.1% of non-mCRC from C2, and 9.8% of CRC from C3.

Misdiagnosis included 4 mCRCs treated with ICI. At the exome level, reevaluation of the MSI genomic signal using MSIC are detected 100% of cases with true MSI status among C1 and C2. Further validation of MSICare was obtained in CRC tumors from C3, with 96.1% concordance for MSI status. Whereas misdiagnosis with MSISensor even increased when analyzing down-sampled WES data from C1 and C2 with microsatellite markers restricted to the MSK-IMPACT gene panel, particularly in the MSH6-deficient setting, MSICare sensitivity and specificity remained optimal (100%). Similar results were obtained with MSICare after targeted NGS of tumors from C4 with the optimized microsatellite panel MSIDIAG. The authors concluded that in contrast to MSISensor, MSICare performs at least as efficiently as the reference method (MSI PCR) in detecting MSI in CRC regardless of the defective MMR protein under WES and targeted NGS conditions. Limitations include lack of evaluation in noncolorectal cancer types. Larger prospective studies are required to confirm findings in mCRC settings involving ICI treatment.

Minimal Residual Disease Testing for Cancer

Colorectal Cancer and Solid Tumors

Reinert et al. (2019) conducted a prospective, multicenter cohort study consisting of 125 patients with stages I-III CRC. Testing was performed pre-operatively, post-operatively, and in longitudinal follow-up. The mean age of the 130 patients enrolled (the data from 5 were not analyzed) was 67.9 years. Pre-operative testing showed that baseline disease detection of circulating tumor DNA (ctDNA) when compared with CEA (a current method for disease surveillance in CRC) was 88.5% vs. 43.3%. In the post-operative setting, immediately before and after ACT treatment, MRD-positive patients had hazard ratios (HR) for relapse of 7.2 ($P < 0.001$) and 17.5 ($P < 0.001$), respectively, when compared to MRD-negative patients. The study also demonstrated that repeat sampling increases the sensitivity of the test. Of the patients who had recurrence, the test had a sensitivity of 87.5% for relapses at or before the time of radiographic detection. In the post-surgical period, a positive test result without additional treatment was followed by relapse in over 98% of cases. With treatment, 30% of MRD-positive patients cleared their ctDNA and remained MRD-negative and disease free throughout the follow-up period. In contrast, longitudinal CEA analysis identified relapse with a sensitivity of 69% and a specificity of 64%, with no lead time when compared with radiologic imaging. The study determined that MRD status was the only factor significantly associated with relapse-free survival, after adjusting for all other standard clinicopathological factors.

Wang et al. (2019) evaluated if circulating tumor DNA can provide a measurement of disease burden to stratify the risk of recurrence in 58 patients with resected colorectal cancer (stage I, II, or III) during postoperative surveillance. Forty-five patients with negative circulating tumor DNA levels were recurrence-free with a median follow-up of 49 months, and 10 of 13 patients with positive circulating tumor DNA levels relapsed during follow-up. Circulating tumor DNA positivity preceded radiologic or clinical evidence of recurrence in all 10 patients by a median of 3 months. Limitations include a small sample size; and the findings need to be validated in a larger, prospective trial.

MRD in Other Solid Tumor Types

Abbosh et al. (2017) conducted a tumor-specific phylogenetic approach to ctDNA profiling in the first 100 TRACERx (TRAcking non-small cell lung Cancer Evolution through therapy (Rx)) study participants. The authors identified independent predictors of ctDNA release and perform tumor volume limit of detection analyses. Through blinded profiling of post-operative plasma, the authors observed evidence of adjuvant chemotherapy resistance and identify patients destined to experience recurrence of their lung cancer. The study showed that phylogenetic ctDNA profiling tracks the subclonal nature of lung cancer relapse and metastases, providing a new approach for ctDNA driven therapeutic studies.

Christensen et al (2019) addressed the prognostic and predictive impact of ultra-deep sequencing of cell-free DNA in 68 patients with localized advanced bladder cancer before and after cystectomy and during chemotherapy. Patient-specific somatic mutations, identified by whole-exome sequencing, were used to assess circulating tumor DNA (ctDNA) by ultra-deep sequencing of plasma DNA. Plasma samples (n = 656) were procured at diagnosis, during chemotherapy, before cystectomy, and during surveillance. Expression profiling was performed for tumor subtype and immune signature analyses. The authors reported the results as follows: Presence of ctDNA was highly prognostic at diagnosis before chemotherapy. After cystectomy, ctDNA analysis correctly identified all patients with metastatic relapse during disease monitoring (100% sensitivity, 98% specificity). A median lead time over radiographic imaging of 96 days was observed. In addition, for high-risk patients (ctDNA positive before or during treatment), the dynamics of ctDNA during chemotherapy was associated with disease recurrence, whereas pathologic downstaging was not. The authors' analysis of tumor-centric biomarkers showed that mutational processes were associated with pathologic downstaging. However, no significant correlation for tumor subtypes, DNA damage response mutations, and other biomarkers was observed. The authors' results suggest that ctDNA analysis is better associated with treatment efficacy compared with other available methods. Randomized clinical trials should be initiated to determine the clinical impact of ctDNA-stratified therapeutic approaches.

Coombes et al. (2019) studied circulating tumor DNA (ctDNA) profiling for detection of breast cancer recurrence in a multicenter, prospective cohort trial. Blood sampling for ctDNA analysis on 188 patients were collected following surgery

and adjuvant therapy every 6 months for up to 4 years. Following an interim analysis, an interim laboratory assessment of serial plasma was conducted on the first 49 patients, which included 18 relapses and comprised an approximately 1:2 ratio of relapsed to nonrelapsed patients. The cohort comprised three main subtypes: 34 patients with hormone receptor–positive (HR +) and human epidermal growth factor receptor 2-negative (HER2-) tumors, eight (8) patients with HR + and HER2 + tumors, and seven (7) patients with triple-negative breast cancer (TNBC). Whole-exome analysis of the archived primary tumor were performed for each patient for custom assay design. Serial plasma samples were analyzed in a blinded manner. Patient-specific somatic variants were identified by comparison of paired primary tumor and matched white blood cell DNA whole-exome sequencing WES profiles for all 49 patients. Personalized assays targeting 16 variants from primary tumor whole-exome data were tested in serial plasma for the presence of ctDNA by ultradeep sequencing. The authors reported plasma ctDNA was detected in 89% (16 of 18) of the patients who relapsed. By subtype, assay sensitivity was 82% in HR +/HER2-, 100% in HR +/HER2 +, and 100% in TNBC. Two relapsed patients were not detected by ctDNA analysis. The assay achieved 100% specificity, as ctDNA was not detected in any of the 156 plasma samples collected from the 31 patients who did not relapse. All ctDNA-positive patients relapsed within 50 months after surgery, and molecular relapse through ctDNA analysis was detected up to 2 years prior to clinical relapse with a median of 8.9 months. The authors reported detection of ctDNA in 16 of 17 patients with a lead time of up to 2 years prior to distant metastatic relapse demonstrating the assay's ability to predict breast cancer recurrence earlier than imaging, CA 15-3, clinical examination, and liver function tests. In addition to ctDNA detection, circulating tumor cells (CTC) have also shown prognostic significance for detection of preclinical metastases in patients with breast cancer. The authors reported that both ctDNA and CTCs could complement conventional recurrence monitoring tests that have limited specificity and frequently result in considerable anxiety and expensive follow-up testing.

MRD in Monitoring of Therapeutic Interventions

Goldberg et al. (2018) conducted a study on the early assessment of lung cancer immunotherapy response via circulating tumor DNA (ctDNA). ctDNA can be distinguished based on the presence of tumor-specific somatic mutations and is expected to have greater specificity than most serum protein markers. Serial blood samples were collected from patients diagnosed with metastatic non–small cell lung cancer (NSCLC) who received immunotherapy with an anti-DP-1 or anti-PD-L1 drug alone or combined with other immunotherapeutic agents. The treating oncologists were blinded to ctDNA testing results, and ctDNA analysis was performed while blinded to clinical data. Of the 49 enrolled patients with metastatic NSCLC who were receiving immune checkpoint inhibitor therapy, the study focused on 28 patients in whom somatic mutations were identified in baseline plasma. There were 182 serial plasma samples analyzed. The authors reported no significant difference in overall survival between the 28 patients who had detectable ctDNA mutations at baseline and the 21 patients who did not. Comparison of baseline characteristics between these two populations yielded no significant differences other than gender. Quantification of ctDNA was done by determining the allelic fraction of cell-free DNA fragments that harbored cancer-associated somatic mutations by using an assay in which multiplexed PCR amplification products of 43 mutation-prone regions in 24 genes were subjected to ultra-deep next-generation sequencing. The authors also evaluated the timing of ctDNA and radiographic responses reporting a median time to initial ctDNA response of 24.5 days from start of treatment compared to 72.5 days by imaging. The authors also evaluated ctDNA response and survival outcomes noting that ctDNA response was associated with a significantly lower risk of disease progression or death. The study demonstrates that ctDNA can be a clinically informative biomarker to complement imaging monitoring of response in patients receiving immune checkpoint inhibitor therapy for NSCLC. The authors concluded that since ctDNA responses were seen significantly sooner than imaging responses, ctDNA monitoring could provide an early measure of therapeutic efficacy, and patients are likely to have a longer duration of treatment benefit and superior progression-free and overall survival. Study limitations include small sample size and biased study population as the study excluded patients who did not have a detectable mutant ctDNA at baseline.

MRD in Hematopoietic/Hematologic Malignancies

Ching et al. (2020) conducted an analytical evaluation of the clonoSEQ Assay for establishing measurable (minimal) residual disease in acute lymphoblastic leukemia, chronic lymphocytic leukemia, and multiple myeloma on a sample of 115 patients. A subset of 66 clinical samples (21 ALL, 22 CLL, and 23 MM samples) of high disease burdens and high mass of gDNA since the contrived samples generated for these studies required higher volumes and tumor burdens than samples submitted for routine clinical assessment. The samples selected were representative of non-unique clonotype sequences while ensuring that no two samples carried an identical clonal sequence. Cancer clonotype sequences were identified in diagnostic 'ID' samples and then measured in follow-up MRD samples using the clonoSEQ Assay. Genomic DNA was amplified using locus-specific multiplex PCR. The goal of this analysis was to determine the sensitivity and specificity of the clonoSEQ Assay by assessing the limit of detection (LoD), limit of quantitation (LoQ) and limit of blank (LoB), which were required to make sample-level MRD estimates for subsequent evaluation studies. The authors reported the results for LoD and LoQ estimated at 1.903 cells and 2.390 malignant cells, respectively. LoB was zero in healthy donor gDNA. Precision ranged from 18% CV at higher DNA inputs to 68% CV near the LoD. Variance component analysis showed MRD results were robust, with expected laboratory process variations at ≤ 3% CV. Linearity and

accuracy were demonstrated for each disease across orders of magnitude of clonal frequencies. Nucleotide sequence error rates were extremely low. The authors concluded that these studies validate the analytical performance of the clonoSEQ assay and demonstrate a potentially highly sensitive diagnostic tool for selected lymphoid malignancies. Limitations include a high disease burden in identifying disease-associated clonotypes.

Rezazadeh et al. (2024) performed a retrospective analysis of patients who achieved remission with first-line therapy for Mantle Cell Lymphoma (MCL) followed by monitoring of minimal residual disease (MRD) with the clonoSEQ assay, which measures circulating tumor cell DNA. The authors reported a total of 34 patients were studied; 22 remained in continuous remission from MCL, of which 21 remained MRD negative. Several patients had 2 to 4-year intervals without any surveillance imaging, while still getting reassurance every few months from the negative clonoSEQ test, and the patient remained in a deep state of remission. The authors concluded that early detection of MRD enabled them to use pre-emptive rituximab therapy in select patients which converted them to an MRD− state. Furthermore, they concluded that for MCL patients in first remission, performing the clonoSEQ MRD assay every 3 months (in lieu of routine surveillance imaging) appears to be an effective surveillance strategy.

Munshi et al. (2021) conducted a large-cohort meta-analysis on the association of minimal residual disease (MRD) with superior survival outcomes in patients with newly diagnosed multiple myeloma (MM). The analysis consisted of fourteen (14) studies (n = 1273) on the impact of MRD on progression-free survival (PFS) and twelve (12) studies (n = 1,100) on overall survival (OS). The authors reported that the results were specifically in patients who had achieved conventional complete response (CR) in 5 studies for PFS (n = 574) and 6 studies for OS (n = 616). An MRD-negative status was associated with significantly better PFS overall and in studies specifically looking at CR patients. Overall survival was also favorable in MRD-negative patients overall and in CR patients. Tests of heterogeneity found no significant differences among the studies for PFS and OS. The authors concluded that MRD-negative status after treatment for newly diagnosed MM is associated with long-term survival and that these findings provide quantitative evidence to support the integration of MRD assessment as an end point in clinical trials of MM.

Hourigan et al. (2020) studied the impact of conditioning intensity of allogeneic transplantation for acute myeloid leukemia (AML) with genomic evidence of residual disease. Ultra-deep, error-corrected sequencing for thirteen commonly mutated genes in AML was performed on patients' preconditioning blood treated in a phase III clinical trial that randomly assigned adult patients with myeloid malignancy in morphologic complete remission to myeloablative conditioning (MAC) or reduced-intensity conditioning (RIC). The authors reported that there were no detected mutations in 32% of MAC and 37% of RIC recipients. Both groups showed similar three-year overall survival (OS). Those who showed positive for next-generation sequencing (NGS) for a detectable mutagen, relapse and survival were significantly different between the MAC and RIC arms. In multivariate analysis for NGS-positive patients, RIC was significantly associated with increased relapse and decreased OS compared with MAC. AML MRD models also showed benefit for MAC over RIC for patients who tested positive. The authors concluded that this study shows evidence that myeloablative conditioning vs. reduced-intensity conditioning in acute myeloid leukemia patients with genomic evidence of minimal residual disease before allogenic transplantation can result in improved survival. Future studies should confirm the hypothesis that RIC remains a reasonable approach in AML when no genomic evidence of residual disease is detected.

Bassan et al. (2019) conducted a systematic literature review and meta-analysis of minimal residual disease (MRD) as a prognostic indicator in adult B-cell acute lymphoblastic leukemia (B-ALL) to explain the clinical significance of MRD to relapse-free survival and overall survival in precursor B-ALL. A total of 32 full text articles and abstracts were included in this meta-analysis review. The overall results on the clinical outcomes showed improvement in relapse-free survival (RFS) for patients who achieved MRD negativity; the effect was consistent across all studies. Overall survival results showed improvement for patients who achieved MRD negativity, and the results were consistent for favorable results of MRD negativity across all but one study. The authors reported that the studies in this systematic review and meta-analysis represent a broad range of patient sub-groups and treatment regimens, and the findings are generalizable across the B-ALL population. Limitations of the analyses were noted because of the wide range of treatment regimens, follow-up times, and methodologies for assessing MRD. The authors concluded that this systematic review and subsequent meta-analysis support the use of MRD as a prognostic marker in the management of patients with acute lymphoblastic leukemia.

Next-Generation Sequencing (NGS) for Solid Tumors and for Myeloid Malignancies and Suspected Myeloid Malignancies

Freedman et al. (2018) used the National Survey of Precision Medicine in Cancer Treatment data to investigate how 1,281 hematologists/oncologists in the U.S. use NGS to assess the association of test use with oncologist practice characteristics. Overall, 75.6% reported using NGS testing to guide their treatment decisions, which the authors state the oncologists are already confronted with a large volume of genomic information that they need to interpret. Of those oncologists, 34% used NGS to guide treatment decisions for those with advanced refractory disease, 29.1% to determine clinical trial eligibility, and 17.5% to determine off-label use of FDA-approved drugs. The study has several limitations; one

is the cooperation rate being lower than that of previous surveys on genomic and genetic test use, and responders may have differed from non-responders in terms of their genetic testing practices and other characteristics such academic affiliation. There is a need to monitor the use of new technologies to ensure patients with cancer have access to appropriate testing and effective therapies.

Zehir et al. (2017) established a large-scale, prospective clinical sequencing study using a comprehensive assay (MSK-IMPACT) through which data of tumor and matched normal sequence was compiled from a cohort consisting of at least 10,000 patients with advanced cancer and available pathological and clinical annotations. Through this data, the authors identified clinically relevant somatic mutations, novel noncoding alterations, and mutational signatures that were shared by common and rare tumor types. Patients were enrolled on genomically matched clinical trials at a rate of 11%. The authors obtained 12,670 tumors from 11,369 patients for sequencing, and the cohort of successfully sequenced tumors were comprised of 62 principal tumor types and > 300 detailed tumor types, which is representative of the diversity of metastatic solid cancer patients treated. The authors were able to detect important genomic alterations that would have been missed by other approaches. Eighty-one percent (n = 63,184) of all mutations were outside the combined target regions of hotspot panels that are unsuitable for detecting most copy number alterations (CNAs) and rearrangements. In comparison to whole exome sequencing (WES) which typically has limited coverage depth, the authors reported that downsampling their data revealed that at least 9% of all mutations would have been missed by WES, including therapeutically targetable alterations in BRAF, EGFR, and MET. MSK-IMPACT produced more uniform coverage across the most clinically relevant genes and can also detect targetable gene fusions based on the inclusion of breakpoint-containing introns absent from current WES methods. Additionally, the authors reported 69% of somatic mutations detected by MSK-IMPACT were not previously reported in the COSMIC database, and these mutations would have been difficult to distinguish from rare inherited variants without a patient-matched normal. The authors concluded that the study results represent a comprehensive and unique genomic dataset of patients with metastatic cancer and that this dataset will prove a transformative resource for identifying novel biomarkers to inform prognosis and predict response and resistance to therapy.

Jennings et al. (2017) established analytical validation of best practice guidelines of next-generation sequencing-based oncology panels: a joint consensus recommendation of the Association for Molecular Pathology and College of American Pathologists. Targeted NGS methods include four major components: sample preparation, library preparation, sequencing, and data analysis. The guidelines provides consensus recommendations on validation and ongoing monitoring of targeted NGS panels in the clinical setting and covers an NGS platform overview, test design, potential sources of error during NGS assay development, optimal validation of number sampling, minimal depth of sequencing establishment, and quality control metrics implementation.

Lin et al. (2019) performed a study on BRCA reversion mutations in circulating tumor DNA to predict primary and acquired resistance to the PARP inhibitor Rucaparib in high-grade ovarian carcinoma. Targeted next-generation sequencing of circulating cell-free DNA (cfDNA) was conducted on patients from pretreatment and post progression plasma with harmful germline or somatic BRCA mutations treated with the PARP inhibitor rucaparib. The authors reported BRCA reversion mutations were identified in pretreatment cfDNA from 18% of platinum-refractory and 13% of platinum-resistant cancers, compared with 2% of platinum-sensitive cancers. Those without BRCA reversion mutations detected in pretreatment cfDNA had significantly longer rucaparib progression-free survival than patients with reversion mutations. Seventy-eight post progression cfDNA were sequenced to study acquired resistance, which identified eight additional patients with BRCA reversion mutations not found in pretreatment cfDNA. For patients who relapsed from prior chemotherapies and need to urgently find the next treatment options, this type of minimally invasive assay can efficiently detect BRCA reversion mutations that predict resistance to PARP inhibitors and may provide information on tumor heterogeneity.

Alonso et al. (2019) studied the clinical utility of a next-generation sequencing panel for acute myeloid leukemia (AML) diagnostics and hypothesized that a 19-gene AML-targeted NGS panel could be a valid approach in obtaining clinically relevant information by assessing the panel's ability to classify AML patients according to diagnostic and prognostic indexes in a cohort consisting of 162 patients. Three-hundred thirty-nine variants were found (36% INDELs and 64% single nucleotide variants) in 18 of the 19 genes. The AML sequencing panel detected a total of 4,341 variants. There were 64 mutations founds via NGS approach. In comparison to conventional molecular biology techniques (CMBTs) that are used in routine practice, the NGS panel analyzed more hotspots in a single assay with limited amounts of DNA enabling the panel to reveal additional markers used for AML classification, prognosis, or treatment selection; only 45.7% of patients had a molecular marker using CMBT analysis. With NGS, 88.89% showed at least one useful molecular marker. Sixty-eight percent (n = 110) of patients belonged to the category for AML with recurrent abnormalities. There were 18.5% (n = 30) with cytogenetic aberrancies and 49.4% (n = 80) showed molecular changes. Twenty percent (n = 32) were classified as AML with changes related to myelodysplasia and 12% (n = 20) remained in the AML not otherwise specified category. The authors concluded that this study supports that a targeted NGS-based assay is reproducible and precise and is therefore applicable in the diagnostic set for acute myeloid leukemia.

Molecular Biomarkers for Risk Stratification of Indeterminate Pulmonary Nodules Following Bronchoscopy

Lee et al. (2021) studied the impact of the Percepta genomic classifier in a real-world prospective multicenter registry across 35 U.S. centers on clinical management decisions in patients with low- and intermediate-risk lung nodules following a nondiagnostic bronchoscopy. A subset of patients with an inconclusive bronchoscopy for a pulmonary nodule was analyzed, with an adjudicated lung diagnosis with at least a 12-month follow-up. In this cohort, change in the decision to pursue additional invasive procedures following Percepta results was assessed. There were 283 patients who met study eligibility criteria. The authors reported a negative Percepta result that down-classified the risk of malignancy in 34.3% of cases; of these patients, 73.9% had a change in management plan from an invasive procedure to surveillance with the majority avoiding a procedure up to 1 year post initial evaluation. Study limitations include its observational aspect, which allowed for a wide variation in clinical decision-making. Future studies are needed to help further define the clinical utility of Percepta in decreasing invasive procedures intermediate risk patients are down-classified by a negative Percepta result.

Silvestri et al. (2015) conducted a multi-center prospective study (AEGIS-1 and AEGIS-2) to validate a bronchial-airway gene-expression classifier that could improve the diagnostic performance of bronchoscopy for 639 smokers (current or former). A gene-expression classifier measured in epithelial cells was collected from the mainstem bronchus that appeared normal in order to assess lung cancer probability. Forty-three percent of bronchoscopy exams were non-diagnostic for lung cancer. Invasive procedures were performed post bronchoscopy in 35% of patients with benign lesions. The authors reported the following results: In the AEGIS-1 study, the gene-expression classifier had an area under the receiver-operating-characteristic curve (AUC) of 0.78, 88% sensitivity, and 47% specificity. In the AEGIS-2 study, the gene-expression classifier had similar results with an AUC of 0.74, 89% sensitivity, and 47% specificity. The combination of the classifier plus bronchoscopy had a 96% sensitivity of in AEGIS-1 and 98% in AEGIS-2, independent of lesion size and location. In 101 patients with an intermediate pretest probability of cancer, the negative predictive value of the gene-expression classifier was 91% among patients with a non-diagnostic bronchoscopic exam. The authors concluded that the gene-expression classifier improved diagnostic performance of bronchoscopy for lung cancer detection. Study limitations include insufficient or poor-quality RNA, which prevented measurement of the gene-expression classifier. Also, there was 9% attrition, and 5% lacked a definitive diagnosis at 12 months following bronchoscopy. Also, the study lacks generalizability as it focused on smokers and therefore making it unclear whether a similar field of injury exists in nonsmokers, and further studies are needed to evaluate this.

Mazzone et al. (2022) conducted a multicenter prospective study to evaluate the performance of Percepta Genomic Sequencing Classifier (GSC) in risk re-classification of indeterminate lung lesions. The study included patients who currently or formerly smoked and were undergoing bronchoscopy to evaluate lung nodules and were followed for a minimum of 12 months after bronchoscopy. The results were based on 412 patients from the AEGIS cohorts (I and II) and Percepta registry with the most common histological cancer types of adenocarcinoma and squamous cell lung cancer. The authors reported that overall, 27.5% of patients with low- and intermediate-risk lesions that were benign underwent further invasive procedures after a non-diagnostic bronchoscopy. The study showed that down-classification by Percepta GSC can reduce invasive procedures by 50% in this population and that patients with nodules that are down-classified should be managed according to the guideline recommendation for continued surveillance imaging until the nodule is ascertained to be benign. Additionally, Percepta GSC's up-classification of intermediate- and high-risk malignant lesions would have decreased unnecessary diagnostic procedures in approximately 30% of these patients with the potential for an earlier diagnosis. Limitations of the results include a follow-up of only 12 months to determine benign status, which may have contributed to the inability to adjudicate a diagnosis for 45 patients, increasing the true prevalence of malignancy. Further studies on clinical utility is needed to assess the benefits of the classifier in real-world settings.

Pharmacogenomics Testing (PGx)

BCHE, CACNA1S

Borden et al. (2021) evaluated and synthesized pharmacogenomic evidence that may inform anesthesia and pain prescribing to identify clinically actionable drug/gene pairs. There were 93 out of 180 (51%) of commonly used perioperative medications with published pharmacogenomic information; of those, 18 had actionable evidence. A known perioperative pharmacogenetic example is malignant hyperthermia; various genetic polymorphisms in the RYR1 and CACNA1S genes predispose individuals to this life-threatening hypermetabolic response to succinylcholine and certain volatile anesthetics. All articles within a given drug/gene or drug/variant pair group with an existing published clinical pharmacogenomic guideline (CPIC, DPWG) or with pharmacogenomic information in the FDA label were automatically eligible and taken forward for full review, which were then rigorously evaluated for scientific, genetic, statistical, and clinical methodological rigor. A total of 18 medications that were deemed potentially clinically actionable have similar CPIC, DPWG, and/or FDA label prescribing guidance; and clinical decision support (CDS) were developed and subjected to AGREE II scoring. All CDS summaries were unanimously recommended for clinical implementation and thus deemed

clinically actionable. The authors reported their study creates an evidence-driven decision-support framework to examine the potential clinical utility of having pharmacogenomic results for key perioperative medications in advance of a patient's surgery date.

Levano et al. (2017) developed a resequencing DNA array, known as the perioperative patient safety (POPS) array, to screen the entire coding sequences of the RyR1, CACNA1S and BCHE genes for variant detection that are known to contribute to acute, life-threatening risks as seen with malignant hyperthermia (MH) and butyrylcholinestherase (BCHE) deficiency, which are inherited pharmacogenetic disorders of primary interest in perioperative medicine. Patients who are MH-susceptible (n = 121) were identified and genotyped with the arrays using a standard diagnostic tool for MH susceptibility. The authors detected 29 predetermined RyR1 variants in 44 individuals in 97% of the cases, among them all 16 variants of established diagnostic value. In an arrays trial application, 21 MH-susceptible individuals with no known RyR1 or CACNA1S variants were screened, resulting in discovery of new variants, which was confirmed by capillary sequencing. The authors concluded that the arrays offer an efficient high-throughput alternative for diagnostic genotyping of genes affecting MH susceptibility, BCHE deficiency, and other neuromuscular disorders, while enabling a comprehensive search for rare variants in these genes.

CYP2B6

Liu et al. (2022) evaluated genetic variants in CYP2B6 and HSD17B12 associated with risk of squamous cell carcinoma of the head and neck (SCCHN). The authors used a hypothesis-driven approach to comprehensively assess associations of genetic variants in 43 polycyclic aromatic hydrocarbons (PAHs) and tobacco-specific nitrosamines (TSNA) metabolism-related genes and the risk of SCCHN and its subtypes, by regulating corresponding gene expression or affecting mRNA alternative splicing. Two single nucleotide polymorphisms (SNPs) in CYP2B6 were identified in a single-locus analysis that are associated with the risk of SCCHN; two SNPs are associated with oropharyngeal cancer risk; and one SNP is associated with oral cancer risk. Polymorphisms in the CYP2B6 gene have also been associated with the risk of breast cancer and acute myeloid leukemia. The authors concluded that two SCCHN risk-associated SNPs were correlated with mRNA expression levels of the corresponding genes and that two SNPs are located at the potential promoter regions and may regulate mRNA expression and alternative splicing. Further studies on functional validation and population replication are necessary to substantiate the study findings.

Bousman et al. (2023) developed Clinical Pharmacogenetics Implementation Consortium (CPIC) guidelines for certain genotypes such as CYP2B6 impact on and antidepressant dosing, efficacy, and tolerability. A systematic literature review on certain genotypes including CYP2B6 and its influence on antidepressant therapy was conducted. Evidence suggests that CYP2B6 genetic variation is associated with sertraline exposure. Recommendations are provided for using the genotype results to help guide antidepressant selection and dosing. Refer to the Clinical Practice Guidelines below from CPIC for the recommendations on CYP2B6 genotypes and antidepressants.

CYP4F2

Danese et al. (2019) studied the effect of CYP4F2, VKORC1, and CYP2C9 in influencing coumarin dose in a meta-analysis of more than 15,000 individuals to capture the possible effect of ethnicity, gene-gene interaction, or other drugs and to verify if inclusion of CYP4F2*3 variant into dosing algorithms improves the prediction of mean coumarin dose. This individual patient data meta-analysis confirmed findings from the authors' previous study, confirmed in this larger cohort of primary studies, that the estimated effect size was nearly 10%. This study also identified a significant effect on gender that men had a lower effect of the T allele when compared to women. A higher dose of coumarin drugs was needed in carriers of the T allele if they were whites or Asians but not in blacks or in other ethnic groups. This study also identified differences between different coumarin drugs, in particular, patients taking acenocoumarol and carrying the T allele needed a higher dose of the drug when compared with patients taking warfarin and carrying the same polymorphism.

Additionally, the study provides reliable prediction models to assist physicians in estimating the stable dose of warfarin based on genotypes, anthropometric and demographic factors, ethnicity, and the use of other drugs. The utility of these models in clinical practice needs to be established in further RCTs before their widespread utilization in clinical settings.

Tatarunas et al. (2022) studied the impact of CYP2C19 and CYP4F2 variants for bleeding prediction in ST-elevation myocardial infarction (STEMI) patients on ticagrelor, an antiplatelet drug to prevent coronary blood clots post interventional procedures in patients with acute coronary syndromes. The study included 144 consecutive patients admitted with ST elevation myocardial infarction. Blood-direct and real-time PCR were used to detect variants. The median age was 68 years and 52.1% were male. Prior to admission, none of the patients used P2Y12 receptor blockers – antiplatelets or anticoagulants, and 27.1% of patients were already on aspirin. After loading dosages of ticagrelor and aspirin, 36 patients (25%) experienced dyspnea. Stent thrombosis were seen in 11 patients, high on-treatment platelet reactivity in 46 patients, minor in-hospital bleeding in 19 patients, and major in-hospital bleeding in 10 patients. Bleeding

events were determined in 20.1% of the studied patient sample, in total. The authors reported that patients with bleeding events had the CYP2C19 GG (*1*1) variant more frequently than patients without bleeding events. The CYP4F2 TT variant was more frequently detected in patients with bleeding events 3 months after hospitalization. The authors reported that this technique could detect variants of the genes associated with ticagrelor antiplatelet therapy within 40 minutes to facilitate individualized treatment for patients with STEMI. The authors determined that CYP4F2 variants had no significant effect on platelet aggregation. The results of this prospective study demonstrated that platelet count, platelet aggregation level, CYP2C19 and CYP4F2 variants might be useful for the grouping of STEMI patients undergoing PCI into 3 categories (minor, major, or no bleeding risk) during and 3 months after hospitalization. Study limitations include a small sample size, and the study population was limited to only patients with STEMI.

IFNL4

Ramamurthy et al. (2018) studied the impact of interferon lambda 4 genotype on interferon-stimulated gene expression during direct-acting antiviral therapy for Hepatitis C, as a part of the BOSON phase 3 randomized open-label trial. That study determined the safety and efficacy of sofosbuvir antiviral medication with and without Peg-IFN-alfa, in treatment-experienced patients with cirrhosis and Hepatitis C virus (HCV) genotype 2 infection and treatment-naïve or -experienced patients with HCV genotype 3 infection. In the present study, the authors analyzed patients given directly acting antiviral (DAA) therapy without IFN (arms 1 and 2). The authors reported that in patients with cirrhosis, those with IFNL4 CC genotype were significantly more likely to achieve sustained virological response (SVR), but this was not the case for those without cirrhosis. This study analyzed the host response in blood and liver of patients suffering from chronic HCV genotype 3 infection from the BOSON trial, where the selected group that had the lowest response rate to DAA therapy with a 50% SVR was analyzed, and a host response might have the largest impact. Overall, the authors concluded that the data indicates a clear impact of IFNL4 genotype on clinical outcome in patients with cirrhosis treated with a suboptimal DAA regimen, together with a clear association between IFNL4 genotypes and gene expression and suggests that the detected changes in gene expression linked to the IFNL4 region drive favorable outcomes. In a clinical setting where liver biopsy is rarely performed, peripheral blood gene expression studies can provide significant signals to link genotype, gene expression, and clinical response. Addition studies are needed to understand the physiological basis for the powerful impact of cirrhosis on outcome in relation to genotype.

Holzgruber et al. (2024) studied the induction of programmed cell death 1 (PD-1) in melanoma cells via type I interferon receptor (IFNAR) signaling and reversal of immune checkpoint blockade (ICB) efficacy through IFNAR pathway inhibition. The authors analyzed a single-cell (sc) RNA-seq melanoma patient dataset for tumor cell expression of cytokine and growth factor receptors known to regulate the PD-1 pathway in T cells. The authors reported patient melanoma cells expressed significantly higher levels of IFNAR heterodimers, while most other receptors evaluated tended to be more prevalent on T-cells. Treatment of melanoma cells with IFN-α or IFN-β triggers IFNAR-mediated Janus kinase-signal transducer and activator of transcription (JAK/STAT) signaling, increases chromatin accessibility and resultant STAT1/2 and IFN regulatory factor 9 (IRF9) binding within a PD-1 gene enhancer and leads to PD-1 induction. IFNAR1 or JAK/STAT inhibition suppresses melanoma-PD-1 expression and disrupts ICB efficacy in preclinical models. The study results uncover type I IFN-dependent regulation of cancer cell-PD-1 and provide mechanistic insight into the potential unintended ICB-neutralizing effects of widely used IFNAR1 and JAK inhibitors.

NAT, NAT2

Thomas et al. (2022) summarized population pharmacokinetic studies of isoniazid in tuberculosis (TB) patients with a specific focus on the influence of N-acetyltransferase 2 (NAT2) genotype/single-nucleotide polymorphism (SNP) on clearance of isoniazid antitubercular agent. NAT2 genotype is one of the most important covariates influencing isoniazid plasma concentration. A total of 12 articles were included in this study. In the majority of the studies, the disposition of isoniazid was explained using a 2-compartment model with first-order absorption and linear elimination. Significant covariates influencing the isoniazid pharmacokinetics were NAT2 genotype, body weight, lean body weight, body mass index, fat-free mass, efavirenz antiretroviral, formulation, CD4 cell count, and gender. The authors reported a two or three-fold increase in isoniazid clearance for NAT2 rapid acetylators as compared to slow acetylators in the majority of studies conducted in adult TB population. The authors concluded that all of the studies have reported that NAT2 genotype/SNP was a significant covariate affecting the clearance of isoniazid. Further studies exploring the generalizability and adaptability of developed population pharmacokinetics (PopPK) models (a potential tool for the optimization of antitubercular therapy) in different clinical settings are required.

Haroldsen et al. (2015) conducted a phase 1, open-label study assessing the effect of N-acetyltransferase (NAT) phenotype and genotype on the pharmacokinetic (PK) and safety profiles of amifampridine, a nonspecific voltage-dependent potassium channel blocker used for the treatment of Lambert-Eaton syndrome. The study objectives include assessing the safety and tolerability and PK profile of amifampridine and the major 3-N-acetyl metabolite in slow and fast acetylator phenotypes, and correlating the amifampridine PK profile and its major metabolite with phenotypic acetylation

activity and NAT2 gene polymorphism genotype after single and repeat doses of amifampridine phosphate. A caffeine challenge test and NAT2 genotyping were used to delineate subjects into slow and fast acetylators for PK and tolerability assessment of escalating doses of amifampridine. The authors reported that plasma concentrations of the N-acetyl metabolite were about two-fold higher in fast acetylators, and amifampridine achieved steady state plasma levels within one day of dosing four times daily. No accumulation or time-dependent changes in amifampridine PK parameters occurred. Overall, slow acetylators reported 73 drug-related treatment-emergent adverse events versus 6 in fast acetylators. The authors concluded that variations in polymorphic NAT corresponding with fast and slow acetylator phenotypes significantly affects the pharmacokinetic and safety profiles of amifampridine.

NeuroIDgenetix

Bradley et al. (2018) conducted a multicenter, prospective, randomized trial evaluating the effect of pharmacogenetics-guided treatment (experimental group) compared to standard of care treatment (control group) on patients diagnosed with depression and/or anxiety and determine if it can improve patient outcomes by maximizing drug efficacy. This study included enrolled patients from 20 independent clinical sites throughout the US, with treating physicians specializing in psychiatry, internal medicine, obstetrics and gynecology, and family Medicine. A total of 685 patients included in this study were either new to treatment or inadequately controlled with medications and were randomized and allocated to either the experimental or control groups and followed for up to 12 weeks. Study participants were categorized in one of 3 groups: depression (n = 246), anxiety (n = 235), or both depression and anxiety (n = 204). The authors reported depression outcomes to determine whether NeuroIDgenetix-guided treatment improves patients diagnosed with moderate or severe depression at baseline are as follows: remission rates at the 8 week visit for the experimental group with severe depression was 25% (response rate was 55%) and control group was 9% (response rate was 28%). At the 12 week follow-up, remission rate for the experimental group with severe depression was 35% (response rate was 73%) and control group was 13% (response rate was 36%). When both moderate and severe depression patients were included in the analysis, 8 week response rates were 49% for the experimental group and 41% for the control group. The 12 week follow-up visit response rates were significantly higher for patients in the experimental group (64%) compared to the control group (46%). The authors found that this study showed no significant improvement in patients with mild depression, which aligns with research suggesting anti-depressants may not benefit patients having mild depression. For anxiety outcomes, the authors reported reduction in anxiety in the experimental group compared to the control group. The authors concluded that this study demonstrates NeuroIDgenetix® test improves outcomes for patients with both depression and anxiety in a diverse set of clinical settings.

Olson et al (2017) conducted a prospective, randomized study of 237 patients at an outpatient community-based psychiatric practice evaluating the effects on outcomes for patients diagnosed with neuropsychiatric disorders of pharmacogenetics (PGx)-guided treatment compared to usual standard of care. This study included assessment of patients diagnosed with moderate to severe depression, anxiety, ADHD, and psychotic disorders, which demonstrates the benefits of PGx-guided pharmacotherapy for a range of neuropsychiatric disorders. The authors reported that 53% of patients in the control group had at least 1 adverse drug event compared to 28% of patients with PGx-guided medication management. Neuropsychiatric Questionnaire (NPQ) and Symbol Digit Coding Test (SDC) scores improved for both groups, but there was no statistical difference in efficacy as measured by these assessments within the 90-day observation period. The authors reported that this study demonstrates a statistically significant improvement in adverse drug events (ADE's) with the incorporation of PGx information into the medication management of a variety of psychiatric disorders, and at the same time, maintaining treatment efficacy. The authors found that there was a clear improvement in ADE's and no significant difference in efficacy and also aligns with FDA drug label warning regarding tolerability versus efficacy. The authors concluded that pharmacogenetic testing may facilitate psychiatric drug therapy with greater tolerability and similar efficacy compared to standard of care.

Phenotypic Biomarker Detection from Circulating Tumor Cells

Jaeger et al. (2017) conducted a study analyzing the HER2 status of circulating tumor cells (CTCs) in HER2-positive primary breast cancer patients at the time of diagnosis to assess potential discordance of HER2 status between primary tumor and CTCs, which may have important implications for HER2-targeted therapy use. There were 642 patients in this study, of which 258 (40.2%) were positive for CTCs; 149 of these patients had at least one CTC with strong HER2 staining not associated with tumor size, histological grading, hormone receptor status, or involvement of axillary lymph nodes. The authors reported that 83 (32.2%) of the patients who were CTC-positive had strong HER2 staining, while 31 (12%) had CTCs with negative HER-2 staining, and within-sample variation in the HER2 staining intensities of CTCs was observed in 86 patients. Additionally, pre-menopausal patients were more likely to have HER2-positive CTCs than post-menopausal patients, and patients with ductal or other types of invasive breast cancer were more likely to have HER2-positive CTCs than patients with the lobular type of breast cancer. This shows considerable discordance between the HER2 status of the primary tumor and CTCs in early breast cancer. The authors reported that the HER2 status of CTCs may differ from the HER2 status of the primary tumor in patients with early HER2-positive breast cancer and suggest that

HER2 status needs to be reassessed to correctly to determine the appropriate use of HER2-targeted therapy. There are ongoing trials evaluating the phenotype and molecular characterization of CTCs as a basis for decision-making in targeted drug development and individualized patient treatment.

Müller et al. (2021) conducted a large multicenter analysis assessing the prognostic relevance of circulating tumor cells HER2 status in metastatic breast cancer (MBC) patients screened for participation in the DETECT study program. Analysis of screening blood samples were done on 1,933 patients with HER2-negative MBC, with HER2 status determined in tumor tissue. The HER2 status of the primary tumor was available in 1,660 patients and of metastatic tissue in 1,061 patients, and the median age was 62 years. Of these, 102 were enrolled in the DETECT III trial, and 213 were enrolled in the DETECT IV trial. A total of 1,217 out of the 1,933 screened patients (63%) detected ≥ 1 CTC per 7.5 ml blood; ≥ 5 CTCs were detected in 735 patients (38%). CTC HER2 status was assessed in 1,159 CTC-positive patients. Strong staining intensity was seen in 2%, intermediate staining intensity in 6%, and weak staining intensity in 15%, whereas 77% were HER2 negative. At least one CTC with strong HER2 staining was found in 174 (15.0%) patients, and 408 patients (35.2%) had at least one CTC with moderate or strong staining. The authors reported that overall survival (OS) was significantly associated with CTC status: median OS in those with ≥ 1 CTC was 15.5 months compared with 37.2 months without CTC and 12 months with ≥ 5 CTCs compared with 28.6 months with < 5 CTCs. The authors concluded that CTC detection in HER2-negative patients is a strong prognostic factor. The presence of ≥ 1 CTC with strong HER2 staining was associated with shorter overall survival, which supports a biological role of HER2 expression on circulating tumor cells.

Armstrong et al. (2019) conducted a prospective, multicenter, blinded validation study of men with high-risk metastatic castration-resistant prostate cancer (mCRPC) and evaluated the ability of baseline (pretreatment) AR-V7 status in circulatory tumor cells (CTCs) to predict treatment outcomes. There were 118 men enrolled in the PROPHECY trial who were starting abiraterone or enzalutamide treatment for prostate cancer treatment. The primary endpoint was progression-free survival (PFS) with the primary objective of validating that AR-V7-negative patients have prolonged PFS with either medication compared with AR-V7-positive patients at the trial level. Overall survival (OS) and response rates were secondary clinical end points. The authors reported 55 men were treated with abiraterone, 58 were treated with enzalutamide, and five received both therapies concurrently. The median follow-up time was 19.6 months among surviving patients, with 102 PFS events and 53 deaths at the primary end point. Median PFS was 5.8 months, and median OS was 20.3 months, reflecting the high-risk features of this population. Majority of CTCs in men with mCRPC were AR-V7 negative; even in AR-V7–positive patients, the proportion of AR-V7–positive cells ranged from 1% to 100%. At progression on abiraterone or enzalutamide, Epic Sciences criteria detected 14 (20%) of 69 evaluable men, and the Johns Hopkins University criteria detected 26 (34%) of 77 evaluable men had AR-V7 detection, which suggests the induction or selection of AR-V7 expression. In this study, the authors demonstrated that men with high-risk mCRPC who are AR-V7 positive by either of two different assays have little evidence of clinical benefit from abiraterone or enzalutamide treatment, a very low probability of confirmed PSA decline, and a short overall survival and progression-free survival. PSA declines with abiraterone or enzalutamide are associated with improved PFS and OS, which supports PSA monitoring for AR-V7–negative men with mCRPC. AR-V7 likely explains up to 25% of AR therapy resistance, implying that most treatment resistance mechanisms remain unidentified. Study limitations include the lack of testing with alternative treatment strategies (e.g., docetaxel chemotherapy) in AR-V7-positive men with mCRPC. Testing patients with multiple poor-risk prognostic features similar to those included in our study could therefore inform the decision to proceed with hormonal therapy or docetaxel chemotherapy. AR-V7–positive men with mCRPC still have a reasonable probability of response and clinical benefit with chemotherapy. Therefore, the study results will inform clinical practice given the confirmed low probability of benefit with current AR inhibitors in AR-V7–positive men, in particular for those who were previously exposed to potent AR inhibitors. The authors concluded that AR-V7 is a strong predictor of clinical outcomes in men with mCRPC treated with abiraterone or enzalutamide.

Armstrong et al. (2020) conducted a prospective multicenter study of circulating tumor cell AR-V7 and taxane versus hormonal treatment outcomes in poor-risk metastatic castration-resistant prostate cancer (mCRPC). The purpose of this study is to assess the benefits from taxane chemotherapy for men with mCRPC treated with enzalutamide or abiraterone. The authors reported the final results of the .PROPHECY trial where this study population were observed longitudinally with CTC AR-V7 testing performed before androgen receptor (AR) inhibitor treatment and again at AR therapy progression before taxane chemotherapy and observed for long-term clinical outcomes. The authors reported that 51 of 118 treated with subsequent AR inhibitor therapy experienced progression and were treated with taxane chemotherapy, including docetaxel (n = 42) or cabazitaxel (n = 9). Median follow-up times from study registration (before abiraterone or enzalutamide treatment) and taxane initiation were 35 and 23 months, respectively. Of the PROPHECY cohort of 118 men, 105 experienced progression during abiraterone or enzalutamide treatment, of which 101 were radiographic or clinical progression events and four were based on rapid PSA rises that led to a change in systemic therapy; 92 men (78%) died. There were 50 of the 51 men treated with taxane chemotherapy experienced progression, based on radiographic or clinical progression in 48, and 41 died. The authors concluded that the men with CTC AR-V7–positive

disease who experience progression with abiraterone or enzalutamide still derive similar benefits from subsequent taxane chemotherapy, suggesting that AR signaling inhibitor therapy should not be offered to those who test positive for AR-V7 by either assay. Limitations include a small sample size and a lack of randomization to taxane or AR therapy based on AR-V7 test results, and the results show a prognostic rather than predictive utility. The PROPHECY data further support CTC AR-V7 status as an important clinically useful biomarker for men with poor-prognosis mCRPC and are facing a decision on further AR-targeted or taxane therapy. Larger controlled prospective studies that more comprehensively assess CRPC genotypes, phenotypes, and AR splice variants are required to confirm the predictive utility of CTC AR-V7 for patient and tumor genomic factors.

Predictive Classifiers for Early-Stage Non-Small Cell Lung Cancer (NSCLC)

Woodard et al. (2021) evaluated 250 stage I-IIA NSCLC patients who underwent molecular risk stratification with a clinically-certified 14-gene prognostic assay. Molecular high-risk (MHR) patients were prospectively advised to undergo adjuvant platinum-based chemotherapy (AC). Differences in freedom from recurrence (FFR) and disease-free survival (DFS) were evaluated. The authors reported the following results: at median follow-up of 29 months, prospective molecular testing yielded an estimated FFR of 94.6% and 72.4% in low-risk and untreated MHR patients, and 97.0% among MHR patients receiving AC. However, there was no association between EGFR status and recurrence, while molecular risk predicted survival and response to AC within both the EGFR mutation(+) and mutation(-) populations. There were 67% of EGFR(+) and 49% of EGFR(-) patients who were molecular low-risk. The authors concluded that this study demonstrates the utility of the 14-gene assay independent of EGFR mutation. Basing adjuvant intervention in early-stage NSCLC on EGFR status alone may undertreat up to 51% of EGFR(-) patients likely to benefit from adjuvant intervention, and overtreat as many as 67% of EGFR(+) patients more likely to be free of residual disease. Limitations of this study include a non-randomized distribution of MHR patients between adjuvant therapy and observation. Further studies are needed on the long-term benefit of third generation tyrosine kinase inhibitors (TKIs) in the adjuvant setting; the combined use of molecular risk stratification and EGFR mutation analysis may help define optimal treatment strategy for all patients after an attempt at curative resection of NSCLC.

Woodard et al. (2018) conducted an observational prospective study using the Razor 14-Gene Lung Cancer Assay that included 100 consecutive patients with stages IA, IB, and IIA nonsquamous NSCLC treated with a surgical resection that received molecular testing for risk stratification. The sample had a median age of 67.7 years and was composed of 58 Stage IA, 32 Stage IB, and 10 Stage IIA patients. The treating clinicians were made aware of the results of the molecular classification results, though the decision of whether or not a patient received adjuvant chemotherapy was individualized to the patient. There were 52 patients stratified as molecular low-risk, and 48 stratified as molecular high-risk, which for this study included both intermediate and high-risk classifications. No patients with molecular low-risk disease were given adjuvant treatment. The 5-year disease free survival was 93.8% among those with molecular low-risk disease and 91.7% among those with molecular high-risk disease treated with adjuvant chemotherapy. For those with molecular high-risk disease not treated, the 5-year disease-free survival was 48.7%. Study limitations include a lack of randomization to adjuvant chemotherapy, a short median follow-up of 23 months, and a small patient sample size, which contributed to a limited number of recurrence events, and therefore a low statistical power. The authors concluded that despite these limitations, they observed significantly better disease-free survival (DFS) in molecular high-risk patients who elected to be treated with adjuvant chemotherapy.

ProMark Risk Score

Blume-Jensen et al. (2015) conducted a study on the development and clinical validation of an in situ biopsy-based multimarker assay for risk stratification in prostate cancer. Two independent clinical biopsy studies included a biomarker assay development cohort (n = 381) separate from the validation cohort (n = 276). The biomarker assay development cohort is a noninterventional, retrospective clinical assay development study using biopsy case tissue samples to define the best marker subset from those shown to correlate with both prostate pathology aggressiveness and lethal outcome; the goal was to define a model that would distinguish between prostate pathology usually recommended for active surveillance (G6). The clinical validation cohort is a noninterventional, blinded, prospectively designed, retrospectively collected clinical study validating the performance of an 8-biomarker assay to predict prostate pathology on its own and relative to current systems for patient risk categorization using biopsy samples from a patient cohort independent of the one used for assay development and generated a risk score for each sample. The authors reported the results as follows: a favorable biomarker risk score of ≤ 0.33, and a nonfavorable risk score of > 0.80 were defined on "false-negative" with a rate of 10% and "false-positive" rate of 5%. At a risk score ≤ 0.33, predictive values for favorable pathology in very low-risk was 95%, low-risk NCCN was 81.5%, and low-risk D'Amico group was 87.2%, higher than for the current risk classification groups at 80.3%, 63.8%, and 70.6%, respectively. Nonfavorable pathology predictive value was 76.9% at biomarker risk scores > 0.8 across all risk groups. Across all risk groups, increased biomarker risk scores correlated with decreased frequency of favorable cases. The validation study met the two coprimary endpoints, separating favorable from nonfavorable pathology and GS-6 versus non-GS-6 pathology. The authors concluded that the 8-biomarker assay

provided individualized, independent prognostic information relative to the current risk stratification systems at the time of this study making it a potentially useful addition to improve the accuracy of decision-making in clinical practice.

Shipitsin et al. (2014) developed a performance-based strategy to identify protein biomarkers predictive of prostate cancer aggressiveness and lethality regardless of biopsy-sampling variation. In this study, expert pathologists blindly assessed prostatectomy samples from a large patient cohort with long follow-up and identified the tissue regions with the highest and lowest Gleason grade from each patient. A core from a high-Gleason and low-Gleason area from each patient sample was used to generate a high and a low tumor microarray to simulate biopsy-sampling error. The authors identified 12 biomarkers from 160 candidates using a quantitative proteomics approach that predicted prostate cancer aggressiveness (surgical Gleason and TNM stage) and lethal outcome in both high-Gleason and low-Gleason areas. This proteomic approach involves measuring proteins from only the tumor region of intact tissue and can possibly improve accurate risk classification at the biopsy stage. Study limitations include the fact that the biomarkers were analyzed in multiple ways for uni- and multi-variate performance on a single, large cohort and increases the risk of the results being over-fitted to patients in this study. Further studies of the 12 biomarkers in this study would enable development of an objective clinical biopsy test based on multiplex proteomics in situ imaging.

Prognostic and Predictive Molecular Classifiers for Bladder Cancer

Seiler et al. (2017) investigated the ability of molecular subtypes of muscle-invasive bladder cancer (MIBC) by gene expression to predict pathological downstaging and survival after neoadjuvant chemotherapy (NAC). Whole transcriptome profiling was performed on pre-NAC transurethral resection specimens from 343 patients with MIBC. Samples were classified according to four published molecular subtyping methods. The authors developed a single-sample genomic subtyping classifier (GSC) to predict consensus subtypes classified as: claudin-low, basal, luminal-infiltrated and luminal, with the highest clinical impact in the context of NAC. Overall survival (OS) according to subtype was analyzed and compared with OS in 476 non-NAC cases. The subtypes were assigned by gene expression analysis. The authors reported that the models generated subtype calls in expected ratios with high concordance across subtyping methods. GSC was able to predict four consensus molecular subtypes with a 73% accuracy, and clinical significance of the predicted consensus subtypes could be validated in independent NAC and non-NAC datasets. Luminal tumors had the best OS with and without NAC. Claudin-low tumors were associated with poor OS regardless of treatment regimen. Basal tumors showed the most improvement in OS with NAC compared with surgery alone. The study limitations are its retrospective design, and that the analysis was confounded by comparisons between patient cohorts from various studies.

Lotan et al. (2022) compiled a multicenter cohort of bladder cancer patients to evaluate the impact of molecular subtyping on survival, specifically comparing patients who received neoadjuvant cisplatin-based chemotherapy (NAC) followed by radical cystectomy (RC) to those who had RC alone. A combined effort of 4 cohorts was conducted on patients with bladder cancer who underwent RC and had genomic data available from transurethral resection of bladder tumor samples. The authors reported a total of 601 patients with muscle-invasive bladder cancer (MIBC) were studied, of whom 247 had been treated with NAC and RC, and 354 underwent RC without NAC. With NAC, the overall net benefit to overall survival (OS) was 7%, and cancer-specific survival at 3 years was 5%. Nonluminal tumors had the greatest benefit from NAC, with 10% greater OS at 3 years (71% vs 61%), while luminal tumors had minimal benefit (63% vs 65%) for NAC vs non-NAC. The authors concluded that for patients with muscle-invasive bladder cancer, based on whole transcriptome profiling, molecular subtyping assay revealed that nonluminal tumors received the greatest benefit from NAC, whereas patients with luminal tumors experienced a minimal survival benefit. Study limitations include its retrospective design with risk for selection bias.

Solid Organ Allograft Rejection

Bloom et al. (2017) conducted a prospective observational study (DART) on renal transplant patients enrolled within 3 months of transplant and/or at the time of a clinically indicated renal biopsy from 14 clinical sites. After transplant, blood was sampled at scheduled visits. Donor-derived cell-free DNA (dd-cfDNA) was measured using a targeted next-generation sequencing assay that employs 266 single-nucleotide polymorphisms to accurately quantify this measurement in transplant recipients without the need for separate genotyping of the recipient or donor. Plasma levels of dd-cfDNA were measured in 102 kidney recipients, and the levels were correlated with allograft rejection status determined by histology in 107 biopsy specimens. The dd-cfDNA level discriminated between biopsy specimens showing any rejection (T cell–mediated rejection or antibody-mediated rejection [ABMR]) and controls (no rejection histologically). The authors reported the positive predictive value at 61% and negative predictive value at 84% for (AR) active rejection at a cutoff of 1.0% dd-cfDNA. The area under the curve (AUC) for discriminating samples with and without ABMR was 0.87. Positive and negative predictive values for ABMR at a cutoff of 1.0% dd-cfDNA were 44% and 96%, respectively. Median dd-cfDNA was 2.9% (ABMR), 1.2% (T cell–mediated types ≥ IB), 0.2% (T cell–mediated type IA), and 0.3% in controls. The authors concluded that dd-cfDNA may be used to assess allograft rejection and injury, and dd-cfDNA levels < 1% reflect the absence of active rejection (T cell–mediated type ≥ IB or ABMR), and levels > 1% indicate a probability of active

rejection. The authors summarized that this study sets the initial foundation for dd-cfDNA performance characteristics in detecting renal allograft active rejection and injury beyond serum creatinine and without the need for a biopsy.

Richmond et al. (2020) conducted a multicenter, prospective, blinded study (DNA-Based Transplant Rejection Test, DTRT) to examine the relationship between donor fraction (DF) and cardiac allograft rejection. This study included a total of 241 heart transplant patients from 7 centers. Age at transplant ranged from 8 days to 73 years, with 146 subjects < 18 years and 95 ≥ 18 years. Follow-up was at least 1 year, and blood samples drawn at routine and for-cause biopsies. A total of 624 biopsy-paired samples from 174 subjects were analyzed through a commercially available cfDNA assay. A blinded analysis of repeated measures compared the outcomes using receiver operating characteristic (ROC) curves. All primary clinical end-points were monitored at 100%. The authors reported that donor fraction in acute cellular rejection (ACR) 1R/2R (n = 15) was higher than ACR 0R (n = 42). DF in antibody-mediated rejection pAMR1 (n = 8) and pAMR2 (n = 12) were higher than pAMR0 (n = 466). An ROC analysis determined optimal DF threshold, which ruled out the presence of either ACR or antibody-mediated rejection. The authors concluded that a low DF correlates with a high negative predictive value for rejection on endomyocardial biopsy (EMB). Further studies are needed and are currently underway for larger numbers of higher grades of rejection.

Keller et al. (2022) conducted a multicenter, retrospective, observational cohort study on lung transplant recipients within 3 years of transplant at 4 centers between March 24, 2020, and September 1, 2020, which coincided with the onset of the COVID-19 pandemic in the United States. In order to mitigate the risk of COVID-19 infection among lung transplant patients and providers, the transplant centers in this study implemented a home-based surveillance program using plasma dd-cfDNA in preference to traditional surveillance bronchoscopy. Dd-cfDNA was used to detect acute lung allograft dysfunction (ALAD), which is a composite endpoint of acute rejection and infection. ALAD patient levels of dd-cfDNA were compared to stable patients. Performance characteristics of dd-cfDNA ≥ 1.0% to detect ALAD were estimated. The authors reported that a total of 175 patients underwent 380 dd-cfDNA measurements, with 290 of them for routine surveillance purposes. Patients with ALAD had higher dd-cfDNA than stable patients. The estimated sensitivity of dd-cfDNA ≥ 1% was 73.9%, specificity of 87.7%, positive predictive value of 43.4% and negative predictive value of 96.5%, as an indication of underlying ALAD during surveillance testing. The authors concluded that dd-cfDNA identified acute lung allograft dysfunction in asymptomatic lung transplant patients who may not have been identified by only using a clinically indicated biopsy strategy, and the use dd-cfDNA for surveillance screening may demonstrate good performance characteristics for the detection of acute rejection or infection. Further studies are needed to thoroughly assess the differences in dd-cfDNA values and performance characteristics between single vs double lung transplant patients.

Molecular Testing for Risk Stratification of Thyroid Nodules

Silaghi et al. (2021) conducted a systematic review and meta-analysis on Thyroseq v3, Afirma GSC, and microRNA panels compared to previous molecular tests in the preoperative diagnosis of indeterminate thyroid nodules. This study further analyzed the impact of noninvasive follicular thyroid neoplasm with papillary-like nuclear features (NIFTP) reclassification and Bethesda cytological subtypes on the performance of molecular tests. The authors reported a total of 40 eligible studies were included with 7,831 indeterminate thyroid nodules (ITNs) from 7,565 patients. Thyroseq v3 showed the best overall performance, followed by Afirma GSC, and Thyroseq v2. The “rule-out” abilities for Thyroseq v3 surpassed Afirma GEC. Thyroseq v2 and v3 achieved superior “rule-in” properties compared to Afirma GSC. Evidence for Thyroseq v3 seems to have higher quality, aside from the paucity of studies. The performance of Afirma GEC and Thyroseq v2 have been affected by Noninvasive Follicular Thyroid Neoplasms with Papillary-like Nuclear Features (NIFTP) histological reclassification.

Lee et al. (2022) performed a systematic review and meta-analysis to evaluate the diagnostic performance of the second-generation molecular tests in the assessment of indeterminate fine-needle aspiration (FNA) biopsy results. The study included Bethesda categories III and IV, use of Afirma GSC, Thyroseq v3, ThyGeNext index test, and conclusive histopathological results. The study included 15 articles, and the authors reported the following results: pooled data for gene sequencing classifier (GSC) studies on 472 thyroid nodules showed a 96.6 sensitivity, 52.9% specificity, 63% positive predictive value (PPV), and 96% negative predictive value (NPV). Pooled data for ThyroSeq studies on 530 thyroid nodules showed a 95.1% sensitivity, 49.6% specificity, 70% PPV, and 92% NPV. The authors concluded the high sensitivity and high NPV in GSC and Thyroseq v3 have potential to help rule out malignancy among thyroid nodules with indeterminate cytology results. There was no statistically significant difference in diagnostic performances of the two tests indicating that either test is appropriate to determine the malignancy of thyroid nodules. A limitation at the time of this study includes a paucity of published literature evaluating these molecular tests in large cohort analyses. Further long-term outcome data are warranted to make a clear recommendation.

Gene Expression Profile Tests for Decision-Making in Castration Resistant and Metastatic Prostate Cancers

Hamid et al. (2021) conducted a study on patients enrolled in the CHAARTED phase III trial, which established androgen-deprivation therapy (ADT) plus docetaxel (D) as a standard for metastatic hormone-sensitive prostate cancer (mHSPC) based on meaningful improvement in overall survival (OS). The authors of this study performed whole transcriptomic profiling on primary prostate cancer (PC) tissue on enrolled patients prior to systemic therapy. The primary outcome was OS; the secondary outcome was time to castration resistant prostate cancer (ttCRPC). The authors reported that the analytic cohort of 160 patients demonstrated significant differences in transcriptional profile compared with localized PC, with a predominance of luminal B (50%) and basal (48%) subtypes, lower androgen receptor activity (AR-A), and high Decipher risk disease. Luminal B subtype was associated with poorer prognosis on ADT alone but benefited significantly from ADT + D, as opposed to basal subtype which showed no OS benefit, even in those with high-volume disease. Higher Decipher risk and lower AR-A were significantly associated with poorer OS in multivariable analyses (MVA). Additionally, higher Decipher risk showed greater improvements in overall survival with androgen-deprivation therapy plus docetaxel. Small sample size is a study limitation due to the availability of specimens.

Feng et al. (2021) examined whether molecular subtypes predict response to apalutamide treatment in nonmetastatic castration-resistant prostate cancer (nmCRPC), using 233 archived primary tumor samples from the SPARTAN randomized, double-blind, phase 3 trial. Patients in the study were randomized (2:1) to apalutamide with androgen deprivation therapy (apalutamide with ADT) or to placebo with ADT. Patients were stratified into high-risk and low-risk categories for developing metastases based on genomic classifier (GC) scores for high (GC > 0.6) and low to average (GC ≤ 0.6) and into basal and luminal subtypes; associations between these molecular subtypes and metastasis-free survival (MFS), overall survival (OS), and progression-free survival 2 (PFS2) were evaluated. The study included 233 patients with a median age of 73 years. The authors reported the results as follows: A total of 116 of 233 patients (50%) in the SPARTAN biomarker subset had high GC scores. All patients who received apalutamide + ADT had improved outcomes. High GC scores were associated with the greatest improvement in metastasis-free survival (MFS), overall survival (OS), and progression-free survival 2 (PFS2) versus placebo + ADT. Of the 233 patients, 152 (65%) had the basal molecular subtype. There were no significant differences in MFS, PFS2, or OS between patients with the luminal vs basal subtype in the placebo + ADT arm. Those with the luminal subtype in the apalutamide + ADT arm had a significantly longer MFS compared with patients with basal subtype. Similar trends were observed for OS and PFS2. In a regression analysis, the luminal-basal subtype score was significantly associated with MFS in those receiving apalutamide+ADT, while the GC score was significantly associated with MFS in placebo + ADT recipients. The authors concluded that the results suggest GC score and basal-luminal subtype from archived tumor specimens may be biomarkers of response to apalutamide plus ADT in the nmCRPC setting. Higher-risk and luminal subtypes appeared to benefit most. Genomic classifier scores may be useful for identifying patients for early treatment intensification with apalutamide. Basal-luminal subtyping may be a beneficial approach for patient selection for further treatment intensification in trials combining novel therapies with apalutamide. Limitations of the study include its retrospective analyses of samples.

Coleman et al. (2022) evaluated therapeutic implications for intrinsic phenotype classification of metastatic castration-resistant prostate cancer (mCRPC) using RNAseq, digital spatial profiling, and histological assessments from metastatic biopsies and patient-derived xenografts to categorize mCRPCs into subtypes defined by the PAM50 breast cancer classification algorithm. The study included 270 mCRPC tumors partitioned into subtypes LumA (42%), LumB (24%), and Basal (34%), based on proliferation rates and androgen receptor (AR) activity. Though phenotypes of most primary tumors and their metastases remained intact despite therapy with androgen-deprivation therapy (ADT), docetaxel and Androgen receptor signaling inhibitors (ARSIs), there were observed instances of discordance in cases where multiple metastatic tumors were acquired. This occurred in 40% of men (n = 23) for who at least one tumor received a discordant classification from other tumors; for a subset of these, the assignment to a particular phenotype lacked confidence. Excluding tumors with lower confidence PAM50 classification (< 0.75) resulted in a 78% concordance across tumors within an individual. A study limitation is the retrospective nature of the PAM50 classification and treatment outcomes.

This clinical evidence review focuses on molecular pathology/genetic testing reported with unlisted codes and whether the current available evidence is sufficient to draw conclusions about improved health outcomes for the Medicare population. Due to low quality evidence and strength of recommendation, the clinical evidence reviewed is insufficient to conclude that the following individual molecular pathology lab tests impact therapeutic decision-making or directly impact treatment, outcome, and/or clinical management in the care of the Medicare member:

BluePrint® Test

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

systemic therapy. Evidence addressing use of Blueprint in conjunction with MammaPrint is insufficient to support clinical utility at this time.

Marin-Liebana et al. (2023) published an initial analysis from the DETERMIND study. DETERMIND is a prospective, open-label, multicenter study evaluating the utility of the MammaPrint/BluePrint (MP/BP) signature related to determining optimal therapy for individuals with operable, clinically high-risk HR +/HER2- early breast cancer (EBC), stage II-IIIA (up to N1) who have received a recommendation for NCT. One hundred sixty-five individuals from 11 centers have been included in this analysis, with data collected at baseline, at the time of MP/BP results and finally at one and three year follow-ups. The first analysis incorporated 99 participants with a median age of 57 years (range 31-85). Ninety-four percent of these were stage II, with 51% cN1. At the time of MP/BP, 37 individuals (37%) were classified as Luminal A, 58 (59%) were Luminal-B, and four presented as a non-Luminal phenotype (3 Basal, 1 HER2). Corresponding with MP/BP results, 44 pts did not receive NCT. In the MP/BP Luminal A group, 35 (95%) did not receive NCT; for 19 of these individuals, it was replaced by NET. Individuals with MP High-Risk results received NCT in 53 cases (85%). MP/BP results significantly increased confidence on the final treatment decision made collaboratively by the treating physicians and participants. The authors concluded that in individuals with clinical high-risk HR +/HER2- EBC, there is a high frequency (35%) of MP/BP Luminal A, who were able to de-escalate NCT. The use of MP/BP also bolstered the decision to administer NCT in the majority (85%) of those with MP High Risk. The authors assert that these findings support the utility of MP/BP in high clinical risk HR +/HER2- EBC to inform neoadjuvant therapy decisions and increase confidence in clinicians and their patients during shared-decision making. The study was sponsored by Agendia, the manufacturer of the MP/BP test, which presents potential bias. Larger, high-quality prospective trials are needed to further validate these findings.

Pellicane et al. (2022) addressed the need for reliable biomarkers to identify individuals with hormone receptor (HR) + HER2- breast cancer tumors who are likely to receive benefit from neoadjuvant endocrine therapy (NET) in a recent observational registry trial of 1091 individuals with early-stage breast cancer. Participants, who were scheduled to receive neoadjuvant therapy, were prospectively enrolled into the Neoadjuvant Breast Registry Symphony Trial (NBRST), sponsored by Agendia. NBRST compared the prognostic value of the 70-gene risk classifier (MammaPrint) and the 80-gene molecular subtyping signatures (BluePrint) with standard pathological classification methods in response to neoadjuvant treatment. The association of these signatures with clinical response and five-years outcome of participants who underwent treatment with NET (n = 67) were evaluated in a sub-analysis. Standard of care genomic testing with MammaPrint and BluePrint was performed, and participants underwent therapy with NET per their physician's discretion. Primary outcome was pathologic partial response (pPR). Secondary outcomes included distant metastasis-free survival (DMFS) and overall survival (OS). The researchers defined clinical benefit as a pPR or stable disease (SD) with use of NET. Of individuals with genomically Luminal A-Type tumors, 94.4% displayed clinical benefit (50.0% pPR and 44.4% SD). Ninety-five percent of individuals with Luminal B-Type tumors exhibited benefit (55.0% pPR and 40.0% SD). At five-year assessment, individuals with genomically Luminal B tumors had substantially worse DMFS (75.6%) than those with genomically Luminal A tumors (91.1%). The trend for OS was similar, but was not significant (81.0% and 91.1%, respectively). The authors concluded that individuals with 70-gene signature low risk results and genomically Luminal A tumors who were treated with endocrine therapy alone have excellent outcomes at five years. In addition, most individuals with genomically-defined Luminal A- and B-type tumors respond well to NET, which suggests NET may be a safe option for treatment; however, those with genomically Luminal B tumors will also need post-operative chemotherapy or CDK4/6 inhibitors to improve their long-term outcomes. The researchers indicate that genomic classification (defined by the combined use of 70- and 80- gene signatures) is prognostic of long-term outcomes and is related to tumor response, supporting the use of these tests in making neoadjuvant treatment decisions in individuals with early-stage HR + HER2- breast cancer. This study was observational and the number of individuals receiving NET was limited, so the sample size was small and prevented further subgroup analyses. In addition, NBRST participants receiving NET instead of neoadjuvant chemotherapy despite features associated with high clinical risk were more likely to be older and postmenopausal. Larger, prospective trials, such as the ongoing FLEX trial, are needed to confirm the findings of this study.

In NBREaST II, a prospective, neoadjuvant study, Göker et al. (2022) measured the treatment response and 5-year survival outcome in the molecular subgroups by combining the MammaPrint and BluePrint. MammaPrint and BluePrint were carried out on 256 individual core needle biopsies (CNB) to quantify chemosensitivity or endocrine sensitivity in the molecular subgroups. The outcomes measured were DMFS, RFS, and BCSS at long-term follow-up. In the group of individuals who received NCT (n = 234), MammaPrint and BluePrint categorized 50 tumors as Luminal A-Type (21%), 110 as Luminal B-Type (47%), 27 as HER2-Type (12%), 47 as Basal-Type (20%). Of individuals that attained a pCR in response to NCT (n = 47), 4% had a MammaPrint Low-Risk result, and 96% had a High-Risk outcome. All BluePrint-defined HER2-Type and Basal-Type tumors had a High-Risk MammaPrint outcome. At five years, DMFS was significantly lower (p = 0.039) in MammaPrint High-Risk tumors (83.8%; 95% CI 77.4–88.6) versus MammaPrint Low-Risk tumors (91.4%; 95% CI 78.6–96.7). Similar outcomes were seen for 5-year RFS; however, not for BCSS. Limitations to the study

include a small sample size, with no differences in 5-year survival when stratifying the cohort into subgroups. The study confirms previous conclusions signifying that MammaPrint and BluePrint can predict chemosensitivity and 5-year results more precisely versus traditional pathological sub-typing, supporting informed decision-making.

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

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

NCCN’s guideline on breast cancer has no information regarding BluePrint testing. (NCCN Breast Cancer, v5.2024)

Molecular Biomarker Testing for Risk Stratification of Cutaneous Squamous Cell Carcinoma

Patients with Cutaneous Squamous Cell Carcinoma (cSCC) stand to benefit from improved risk stratification to identify those at highest risk of poor outcomes, such as metastasis. However, to demonstrate clinical utility in risk stratification, a new biomarker must provide additional risk stratification (clinical validity) to currently available risk stratification measures (i.e., the combination of staging and clinical plus pathological factors) within a guideline-based standard of care therapeutic framework (i.e., NCCN) to meaningfully reclassify patients with respect to clinical decision thresholds in a manner that improves patient outcomes. To date, this has not been demonstrated for the 40-GEP or other similar tests. Furthermore, the NCCN expert panel on cSCC states that molecular tests that offer risk stratification for cutaneous squamous cell carcinoma have not yet shown to have additional prognostic benefit over traditional staging in NCCN "very high-risk" squamous cell carcinoma, the patient group in which additional prognostic stratification would be of potential value. Of note, the work in the below studies was performed prior to introduction of the NCCN very high-risk category in 2021, and only includes the former categorization of “high-risk.” As a result, this is not reflective of the most up-to-date classification. The proposed algorithm, which is not currently part of clinical guidelines, starts with NCCN high-risk cSCC patients who subsequently undergo 40-GEP testing and staging by Brigham and Women’s Hospital/ American Joint Committee on Cancer (BWH/AJCC8) staging systems for management strategy.

Wysong et al. (2021) conducted a prospectively designed biomarker study to develop and validate a gene expression profile (GEP) test for predicting risk for metastasis in localized, high-risk cSCC to improve risk-directed patient management. The primary outcome was a three-year metastasis-free survival (MFS) (regional and distant), and secondary outcome was disease-specific death from cSCC. A discovery cohort (n = 202) was included in this review, and primary cSCC tissue and clinical data were obtained from 23 centers. Deep machine learning was applied to gene expression data from 122 genes passing initial expression thresholds to identify a prognostic signature capable of patient stratification by risk for regional or distant metastasis from primary cSCC tumors. The 40-GEP algorithm selected for validation was comprised of 2 gene expression signatures, (6 control and 34 discriminant genes), with modeling performed using neural networks. This algorithm generated linear scores for probability of metastasis from each signature. Validation included applying the algorithm to 321 primary cSCC cases with and without metastatic events. The validated

40-GEP was used to define 3 risk groups with increasing risk for metastasis from low (class I) to high (class 2A) to highest (class 2B) risk. The 3-year metastasis-free survival rates were 91.4%, 80.6%, and 44.0%, respectively. A positive predictive value of 60% was achieved for the highest-risk group (class 2B), an improvement over staging systems, and negative predictive value, sensitivity, and specificity were comparable to staging systems. Study limitations included potential understaging of cases that could affect accuracy of the metastasis rate. Patients whose risk is either too low or too high to benefit from the 40-GEP are meant to be excluded from testing. Cases excised via MMS had no tissue available for review other than the shave biopsy, therefore there is possibility for underreporting of high-stage features and resultant under-staging.

Farberg et al. (2021) integrate gene expression profiling into the management of high-risk cutaneous squamous cell carcinoma (cSCC) to improve risk-aligned management recommendations. The authors analyzed data from the clinical validation cohort (described by Wysong et al.) on 300 patients who had high-risk cSCC as defined by the NCCN guidelines available at the time of this study. Risk classifications using a validated 40-gene expression profile (40-GEP) test and T stage were applied to NCCN patient management guidelines. Risk-directed patient management recommendations within the NCCN guidelines framework were aligned based on risk for metastasis. The authors reported that of the 300 NCCN high-risk cSCC patients, 159 (53.0%) were 40-GEP Class 1 and AJCC T1-T2, and 173 (57.7%) were Class 1 and BWH T1-2a, indicating low risk for metastasis (suggesting low management intensity). The 40-GEP integration suggested high intensity management for only 24 (8.0%) patients (all Class 2B), and moderate intensity management for the remainder of the cohort. The authors concluded that the 40-GEP test can be integrated within existing NCCN guideline recommendations for managing cSCC patients to help refine risk-directed management decisions. Integration of the 40-GEP test would allow > 50% of this NCCN-defined high-risk cohort to be managed with the lowest intensity recommendations within the broad NCCN guidelines. High intensity management was deemed risk-appropriate for a small subpopulation (8.0%).

HAX1 Gene Sequencing and Panels of Tests That Include the HAX1 Gene

There is inadequate clinical evidence that the HAX1 gene is likely to impact therapeutic decision-making, directly impact treatment, outcome, and/or clinical management in the care of the member.

Donadieu et al. (2011) reported on Kostmann syndrome, which is diagnosed during the neonatal period by a lack of mature neutrophils and frequent infections. The HAX1 gene associated with this disorder would not generally be tested for in the Medicare population, which is predominantly 65 years of age and older.

Serotonin Transporter Genotyping (HTTLPR)/ HTTLPR Gene Testing

Serotonin Transporter genotyping (HTTLPR) has been associated with response to selective serotonin reuptake inhibitors. However, since the literature is conflicting and limited by small sample sizes, there is insufficient evidence to support coverage.

Studies on 5-HTTLP polymorphisms have been done with regard to PTSD, depression, the impact on decision making and moral choices among others (Armbruster et al., 2023; Hu et al., 2024; Tan et al., 2023). Overall, the evidence is conflicting, and further research is needed to elucidate the clinical utility of genetics and psychiatric disorders.

In a 2011 review of the literature, Margoob et al. reviewed the status of evidence for associations between the serotonin gene polymorphism and some common mental disorders such as affective disorders, post-traumatic stress disorder, obsessive-compulsive disorder, suicide, autism, and other anxiety and personality disorders. The results showed that in one study that included structural imaging indicated that patients with the S allele of 5-HTTLPR, suffering from major depression, exhibit smaller hippocampal volumes only when they had a history of emotional neglect in childhood, compared to patients with only the genetic or the environmental risk factor. Another showed the higher activity genotypes are associated with increased incidence of major depressive disorder in the presence of environmental trauma. In contrast, there are a number of studies that show no association or mixed associations between 5-HTTLPR polymorphism and depressive disorders. The serotonin transporter gene promoter polymorphism (5-HTTLPR) has been repeatedly associated with antidepressant response in mood disorder patients, but findings are not consistent across studies. Though many studies suggest a relationship between 5-HTTLPR and psychiatric morbidity, research results are not consistent with such conclusions and that environment and history may play a larger role. The authors concluded that the available studies of psychiatric genetics is inadequate to include genetics in the diagnostic work-up of the psychiatric patient.

Majumdar et al. (2022) used a summary- based statistics approach, to examine the role of serotonin transporter (SLC6A4) promoter VNTR polymorphism (5-HTTLPR) on the susceptibility of neurobehavioral traits and psychiatric disorders using

the largest available samples for genetic studies. The authors found no evidence of an association between the 5-HTTLPR and the broad range of psychiatric, cognitive, and neurological phenotypes examined.

Nuñez et al. (2023) conducted a meta-analysis of studies that examined the association between clinical and genetic risk factors, specifically monoaminergic transporter genetic variation, and treatment emergent mania (TEM) in bipolar disorder (BD). Seven studies that referenced the SLC6A4 5-HTTLPR polymorphism and TEM were included. The results showed a nominal association between the s allele of the 5-HTTLPR polymorphism with TEM in BD. Future research should focus on complete genome-wide approaches to determine genetic variants that may contribute to TEM to inform the development of personalized medicine best practices treating bipolar depression.

KIF6

There is inadequate clinical evidence that the KIF6 gene is likely to impact therapeutic decision-making, directly impact treatment, outcome, and/or clinical management in the care of the member.

Li et al. (2018) conducted a meta-analysis of published studies to evaluate the relationship between KIF6 rs20455 polymorphism and susceptibility to coronary heart disease (CHD). All eligible studies up to October 5, 2016, were identified. Inclusion criteria included a description of KIF6 rs20455 polymorphism in CHD cases and healthy controls; results expressed as odds ratio (OR); and studies with a 95% confidence interval (CI) for OR with sufficient data to calculate these numbers. Studies were excluded when they had no raw data, or were case-only studies, family-based studies, case reports, editorials, and review articles (including meta-analyses). Twenty-eight publications including 50 individual studies were included in this review. There were no significant associations found between KIF6 rs20455 polymorphism and CHD risk. Furthermore, subgroup analyses were performed by ethnicity, source of control. The authors concluded that KIF6 rs20455 polymorphism may not be associated with CHD susceptibility and the findings do not support the clinical utility of KIF6 rs20455 polymorphism testing in the primary prevention of CHD. The authors recommend future well-designed, large-scale, studies to confirm these results.

Claims of a differential response to statin therapy have led to the marketing of KIF6 screening to assess the suitability of statins for individual patients. Hopewell et al. (2010) conducted a study to evaluate the KIF6 Trp719Arg polymorphism (rs20455) effects on vascular risk and response to statin therapy. Participants (n = 18,348) from the Heart Protection Study were randomized into two groups, with one group receiving Simvastatin 40mg daily and the other group receiving a placebo for a period of five years. Both groups received Simvastatin 40mg daily for four to six weeks prior to randomization. Coronary death or nonfatal myocardial infarction was defined as a major coronary event, and major coronary event plus revascularization or stroke was defined as a major vascular event. The KIF6 genotype was not significantly associated, among placebo-allocated participants, with the risks of incident major vascular events, major coronary events, revascularizations, or strokes. Overall, 40 mg Simvastatin daily produced a 42% reduction in low-density lipoprotein cholesterol, which did not differ significantly by KIF6 719Arg carrier status. Proportional reductions in the risk of major vascular events with statin therapy were similar and highly significant across KIF6 genotypes: 23% in carriers (Arg/Arg or Trp/Arg), and 24% in noncarriers (Trp/Trp). A similar lack of interaction was observed for major coronary events, revascularizations, and strokes considered separately. The authors concluded that regardless of KIF6 genotype, statin therapy similarly reduced the number of coronary and other major vascular events and the use of KIF6 genotyping is not warranted for guiding the use of statin therapy.

Iakoubova et al. (2010) performed a genetic study of the randomized, double-blind, placebo-controlled PROspective Study of Pravastatin in the Elderly at Risk trial (PROSPER), aimed at investigating whether statin therapy among the elderly significantly reduced coronary events in carriers but not in noncarriers in those with and without prior vascular disease. Five thousand seven hundred and fifty-two participants of the PROSPER study (2,804 men and 3,000 women) who were at least 70 years old at the time of enrollment were assessed for the effect of Pravastatin compared with placebo on coronary events according to 719Arg carrier status using proportional hazards models. Since benefit from statin therapy in elderly patients has been primarily shown among those with prior vascular disease, analyses in PROSPER patients with prior disease was performed and the findings were that Pravastatin therapy significantly reduced events in 719Arg carriers but not in noncarriers, P = 0.09 for interaction between treatment and carrier status. Among those without prior disease, no significant benefit was observed in either carriers or noncarriers. Among those with prior vascular disease in the placebo arm, Trp719Arg heterozygotes were at significantly greater risk, compared with noncarriers; the HR of 719Arg carriers, compared with noncarriers, was 1.28. The authors concluded significant benefit from Pravastatin therapy was received in the elderly carriers of the KIF6 719Arg variant with prior vascular disease. However, in noncarriers with prior disease and those without prior disease (carriers or noncarriers) no benefit was noted.

LPA-Intron 25 Genotype Test

The evidence is insufficient to support the use of gene expression testing for coronary artery disease. Further studies with a larger number of patients and longer follow-up are needed to determine if these tests provide clinical utility in cardiovascular risk assessment.

Assimes et al. (2016) summarized the evolution and discovery of genetic risk variants for coronary artery disease (CAD) and their current and future clinical applications. In order to maximize the clinical utility of the current knowledge gained, the authors propose future tasks which include the identification of the remaining susceptibility loci for CAD, proving the clinical utility of genetic data in the prevention of CAD, and acquiring a solid appreciation of the cellular and/or extracellular mechanisms responsible for genetic associations observed at the population level. They conclude that extremely large sample sizes are needed for additional discoveries, given the distribution of effect sizes observed to date for both common and rare variants, as well as the estimated proportion of the heritability of CAD explained by these variants to date. In the coming years, the authors suggest that this need could be fulfilled by mega-biobanks to assist in the determination of the clinical utility of genetic risk scores, and to conduct additional, well-powered Mendelian randomization (MR) studies to complement studies published to date.

Wingrove et al. (2008) performed a microarray analysis on 41 patients with angiographically significant CAD and 14 controls without coronary stenosis to identify genes expressed in peripheral blood that may be sensitive to the presence of CAD. A multistep approach was used, starting with gene discovery from microarrays, followed by real-time polymerase chain reaction (RT-PCR) replication. The authors observed that gene expression scores based on 14 genes, independently associated with the presence or absence of CAD, were proportional to the extent of disease burden. This study is limited by its size and retrospective nature. Larger, prospective studies are needed to confirm these initial results.

The U.S. Preventive Services Task Force (USPSTF) recommendations on the use of nontraditional risk factors in coronary heart disease risk assessment do not address genetic/genomic markers (Lin, 2018).

Prometheus IBD sgi Diagnostic Test

There is insufficient evidence to support an indirect chain of evidence for clinical utility due to lack of details about study methodology and lack of replication of the findings. The intended use of this test is to aid healthcare providers in differentiating inflammatory bowel disease (IBD) vs non-IBD, and Crohn's disease (CD) vs Ulcerative Colitis (UC) in a comprehensive blood test. For distinguishing CD from Ulcerative Colitis UC, clinical validity has not been established. No studies examining the clinical utility of IBD sgi Diagnostic® have been identified. Furthermore, there are no US Preventive Services Task Force (USPSTF) recommendations for genetic or molecular testing for IBDs, and no recommendations for multi-marker panels that include genetic tests to facilitate diagnosis or prognosis of CD or UC.

Lawrence et al. (2015) conducted an observational, nonrandomized prevalence study of patients diagnosed with glycogen storage disease (GSD) type Ia who did not manifest symptoms of inflammatory bowel disease (IBD). The Prometheus IBD sgi Diagnostic test was used to predict and differentiate IBD based on an algorithm combining serologic, genetic, and inflammatory markers. There were 50 patients included in the total cohort. The authors reported 11 of the 50 tested positive for IBD; five had a pattern consistent with CD; another five with UC; and one with nonspecific IBD. The prevalence rate within this cohort was 22%. Limitations include a small sample size. The authors concluded that further research is needed to characterize the relationship between IBD and GSD type I.

In a 2012 review of the monograph, Shirts et al. observed that serologic tests for ASCA-IgA, ASCA-IgG, and atypical perinuclear anti-neutrophil cytoplasmic antibody are standard of care in the diagnostic workup of IBD, although not all investigators include these tests in recommended diagnostic strategies. These 3 markers are included in the 17-marker panel. Based on a meta-analysis of 60 studies (total n = 11,608), pooled sensitivity and specificity of the 3-test panel were 63% and 93%, respectively, for diagnosing IBD. Because the product monograph does not include a comparison of the 17-marker panel with the 3- marker panel, incremental improvement in diagnosis with the 17-marker panel is unknown. The authors calculated an area under curve (AUC) for the 3-marker panel of 0.899 and is not substantially different than the AUC of 0.871 for the Prometheus IBD sgi test. The authors concluded that it is not likely that utilization of this panel will be clinically efficient or cost-effective.

SULT4A1 Genetic Testing

SULT4A1 is an enzyme found in the brain that interacts with numerous neuroactive molecules, including some catecholamines and neural steroids. Findings suggest the SULT4A1-1 haplotype of SULT4A1 is associated with enhanced response to antipsychotic drug therapy. There is insufficient evidence to support Sulfotransferase 4A1 (SULT4A1) genotyping to determine medical management, at this time.

Culotta et al. (2020) investigated the role of SULT4A1, which is a brain-specific sulfotransferase highly expressed in neurons and has been implicated in multiple neurodevelopmental disorders, such as Phelan-McDermid syndrome and schizophrenia. The function of SULT4A1 in the mammalian brain is still unclear as no substrate or biological functions have been identified yet.

VEGFR2 Testing

Vascular endothelial growth factor, subtype 2, (VEGFR2) receptor has been found to be oncogenic. Current studies have targeted VEGFR2 as a potential therapeutic option. There is insufficient evidence to establish a clear association between VEGFR2 mutations and treatment response, at this time.

George et al. (2019) evaluated associations between a subset of single nucleotide polymorphism (SNPs) in genes known to be involved in angiogenesis and clinical outcomes by using data from a subset of patients that were enrolled in the S-TRAC trial, which is a randomized, double-blind, phase III trial on patients with loco-regional renal cell carcinoma (RCC) at high risk of recurrence after nephrectomy. Adjuvant sunitinib prolonged disease-free survival (DFS) versus placebo. Blood samples were prospectively collected from individuals (n = 286) in the S-TRAC trial who consented to a molecular profiling study. Samples were genotyped for 10 single nucleotide polymorphisms (SNPs) and one insertion/deletion mutation. Disease-free survival (DFS) was compared for each genotype in sunitinib versus placebo groups and between genotypes within each of three treatment groups (sunitinib, placebo, and combined sunitinib plus placebo). Longer DFS was observed with sunitinib versus placebo for VEGFR1 rs9554320 C/C, VEGFR2 rs2071559 T/T, and eNOS rs2070744 T/T. Shorter DFS was observed for VEGFR1 rs9582036 C/A versus C/C with sunitinib, placebo, and combined therapies, and A/A vs C/C with sunitinib. VEGFR1 rs9554320 A/C versus A/A was associated with shorter DFS in the placebo and combined groups. The authors concluded that in patients with RCC, correlations between VEGFR1 and VEGFR2 SNPs and increased DFS with sunitinib compared to placebo suggest germline SNPs may predict improved outcomes with adjuvant sunitinib. The authors note that additional future studies are needed to confirm possible prognostic and predictive values of these SNPs. Limitations include small sample sizes in some subgroup analyses.

Hagstrom et al. (2014) investigated the pharmacogenetic relationship between the clinical outcomes of anti-VEGF treatment and eight different SNP variations in the VEGF-A and VEGFR-2 genes in 835 Comparison of AMD Treatment Trials (CATT) study participants at 43 CATT clinical centers. Patients with neovascular age-related macular degeneration (nAMD) were genotyped for seven SNPs in VEGF-A (rs699946, rs699947, rs833069, rs833070, rs1413711, rs2010963, rs2146323) and one SNP in VEGFR-2 (rs2071559). Visual acuity (VA), anatomical features of AMD assessed by optical coherence tomography (OCT) and fluorescein angiography (FA), and the total number of injections given in one year were the clinical measures of the treatment response. For each of the measures of VA evaluated, there was no association with any of the genotypes or with the number of risk alleles. Four of the VEGF-A SNPs demonstrated an association with retinal thickness (rs699947, rs833070, rs1413711). However, adjusted p-values for these associations were all not statistically significant. Among the participants in the two PRN groups, no association was found in the number of injections among the different genotypes or for the total number of risk alleles. The effect of risk alleles on each clinical measure did not differ by treatment group, drug, or dosing regimen. The authors concluded that the evidence did not support substantial pharmacogenetic associations between the VEGF-A and VEGFR-2 SNPs and response to anti-VEGF therapy in patients participating in CATT.

Clinical Practice Guidelines

American Society of Clinical Oncology (ASCO)

In 2022, Andre et al. updated ASCO recommendations regarding the appropriate use of biomarker assay results to inform decisions regarding adjuvant endocrine and chemotherapy in early-stage breast cancer. Evidence for these recommendations was based on information from 24 applicable studies (14 randomized controlled trials and 10 prospective-retrospective). The recommendations include the following: Oncotype DX, MammaPrint, BCI (Breast Cancer Index), and EndoPredict may be used to guide adjuvant endocrine and chemotherapy in postmenopausal individuals or individuals over the age of 50 years with early-stage ER +, HER2- breast cancer that is node-negative or with one to three positive nodes.

ASCO-Society of Surgical Oncology Guideline

ASCO developed recommendations for germline testing in patients with breast cancer. BRCA1/2 mutation testing should be offered to all newly diagnosed patients with breast cancer ≤ 65 years and select patients > 65 years based on personal history, family history, ancestry, or eligibility for poly(ADP-ribose) polymerase (PARP) inhibitor therapy. All patients with recurrent breast cancer who are candidates for PARP inhibitor therapy should be offered BRCA1/2 testing, regardless of family history. BRCA1/2 testing should be offered to women who develop a second primary cancer in the ipsilateral or contralateral breast. For patients with prior history of breast cancer and without active disease, testing should be offered to patients diagnosed ≤ 65 years and selectively in patients diagnosed after 65 years, if it will inform personal and family

risk. Testing for high-penetrance cancer susceptibility genes beyond BRCA1/2 should be offered to those with supportive family histories; testing for moderate-penetrance genes may be offered if necessary to inform personal and family cancer risk. Patients should be provided enough pretest information for informed consent; those with pathogenic variants should receive individualized post-test counseling. Variants of uncertain significance should not impact management, and patients with such variants should be followed for reclassification. Referral to providers experienced in clinical cancer genetics may help facilitate patient selection and interpretation of expanded testing, and provide counseling of individuals without pathogenic germline variants but with significant family history.

Clinical Pharmacogenetics Implementation Consortium (CPIC) Guideline

(Bousman et al., 2023) CPIC guidelines were updated expanding the 2015 CPIC guideline for CYP2D6 and CYP2C19 genotypes and SSRI dosing and summarizing the impact of CYP2D6, CYP2C19, CYP2B6, SLC6A4, and HTR2A genotypes on antidepressant dosing, efficacy, and tolerability. A systematic literature review on certain genotypes including CYP2B6 and its influence on antidepressant therapy was conducted. Evidence suggests that CYP2B6 genetic variation is associated with sertraline exposure. Recommendations are provided for using the genotype results to help guide antidepressant selection and dosing. The basis for the recommendations is a grading system of the evidence linking CYP2D6, CYP2C19, or CYP2B6 genotypes to pharmacokinetic variability, dose, and clinical outcomes that indicates a moderate to high quality of evidence for the majority of data including evidence from large population studies identifying relationships across genotype, drug exposure associations, and treatment discontinuation or switching. CYP2B6-sertraline dosing recommendations include no pre-emptive dose increase because of the small increase in metabolism in CYP2B6 ultrarapid metabolizers (UMs) and rapid metabolizers (RMs). Existing data do not support adjusting starting doses for CYP2B6 intermediate metabolizers (IMs), but reduced metabolism may require a slower titration and a lower maintenance dose than normal metabolizers (NMs). CYP2B6 poor metabolizers (PMs) have reduced metabolism and higher elevated plasma concentrations; and therefore, a lower starting dose, slower titration, along with a 25% reduction of standard maintenance doses should be considered. CYP2B6 genotype results may provide the potential benefit of identifying patients who are at an increased risk of experiencing adverse drug reactions or inadequate response to serotonin reuptake inhibitor (SSRI) antidepressant therapy. A potential risk is the missed identification of rare or novel variants that are typically not interrogated on clinically used testing platforms.

European Heart Rhythm Association (EHRA)/Heart Rhythm Society (HRS)/Asia Pacific Heart Rhythm Society (APHRS)/Latin American Heart Rhythm Society (LAHRS)

In an Expert Consensus Statement on genetic testing for cardiac disease, the EHRA, HRS, APHRS and LAHRS (Wilde et al., 2022) address the state of genetic testing for CAD. The major genes associated with prediction of CAD are APOB, LDLR and PCSK9. In recent decades, widespread contribution of polygenic risk has been shown to contribute to CAD susceptibility and novel genetic mechanisms such as clonal hematopoiesis of indeterminate potential (somatic rather than germline) have also been shown to play a role. Research has indicated that genetic predisposition may prove useful for risk prediction related to CAD, but the predictive utility of PRS for CAD are widely debated and as such, are not commonly used in clinical practice today.

European Society for Medical Oncology (ESMO)

In the 2023 clinical practice guidelines for the diagnosis, treatment and follow up of metastatic non-small cell lung cancer, ESMO states that EGFR mutation status should be determined with test methodology to have adequate coverage of mutations in exons 18-21, including those associated with resistance to some therapies.

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

- Validated gene expression profiles may be used to gain additional prognostic and/or predictive information to complement pathology assessment and help in adjuvant chemotherapy decision making [I, A].
- In cases of uncertainty regarding indications for adjuvant chemotherapy (after consideration of all clinical and pathological factors), expression of urokinase plasminogen activator- plasminogen activator inhibitor-1 (uPA-PAI1) [I, A] or gene expression assays, such as MammaPrint [I, A], can be used.
- Expression of uPA-PAI1 or multigene panels, such as MammaPrint, may be used in conjunction with all clinicopathological factors to guide systemic treatment decisions for individuals where these decisions are challenging, such as luminal B-like/HER2- and N0/N1 breast cancer [I, A].

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

National Comprehensive Cancer Network (NCCN)

The NCCN guidelines for uveal melanoma states molecular markers common in uveal melanomas that may have prognostic significance are not often found in conjunctival or cutaneous melanoma. These include chromosomal abnormalities (particularly chromosomes 3 and 8), and mutations in GNAQ or GNA11 (> 80% of uveal cases), BAP1, SF3B1, and EIFAX. (NCCN Uveal Melanoma, v1.2024)

NCCN's guideline on breast, ovarian and/or pancreatic cancer genetic assessment stated that circulating tumor DNA (ctDNA), detected by mutation profile, copy number changes, altered methylation patterns, fragmentation, size alterations or other approaches has application for disease monitoring as well as early detection. The guideline also stated that, for individuals at increased hereditary risk for cancer, use of pre-symptomatic ctDNA cancer detection assays should only be offered in the setting of prospective clinical trials, because the sensitivity, false-positive rates, and positive predictive value of ctDNA tests for early-stage disease, which are needed to derive clinical utility and determine clinical validity, are not fully defined. (NCCN Genetic/Familial High-Risk Assessment: Breast, Ovarian, and Pancreatic, v3.2024)

The NCCN guideline that addresses Lynch Syndrome (LS) recommends universal screening of all colorectal cancers (CRCs) and endometrial cancers to maximize sensitivity for identifying individuals with LS and to simplify care processes. Microsatellite instability (MSI) and immunohistochemistry (IHC) analyses are screening tests that are typically performed on CRC and endometrial cancer tissue to identify individuals at higher risk for having LS as more than 90% of LS tumors are MSI-high (MSI-H) and/or lack expression of at least one of the MMR proteins by IHC although 10 to 15% of sporadic colon cancers exhibit abnormal IHC and are MSI-H most often due to abnormal methylation of the MLH1 gene promoter, rather than due to LS. The guideline also stated that sporadic endometrial cancers may exhibit abnormal MSI/IHC due to abnormal methylation of the MLH1 promoter. (NCCN Genetic/Familial High-Risk Assessment, v2.2023)

In the NCCN breast cancer guidelines, specific recommendations are not made for types of genetic tests, however the guideline states that for systemic therapy for ER and/or PR positive recurrent unresectable (local or regional) or Stage IV disease, knowing PIK3CA or AKT1 activating mutations or PTEN alterations guides targeted therapies. (NCCN Breast Cancer, v4.2024)

The NCCN soft tissue sarcoma guidelines addresses hereditary retinoblastoma, which is caused by a germline mutation in the retinoblastoma tumor suppressor gene (RB1), is also associated with an increased risk for the development of soft tissue sarcoma (STS). Leiomyosarcoma (LMS) is the most frequent STS subtype with 78% of LMS diagnosed 30 or more years after the diagnosis of retinoblastoma. Although patients with radiation therapy (RT) for retinoblastoma are at significantly increased risk of developing STS, the risks of developing STS are also increased in non-irradiated patients as well, indicating a genetic predisposition to STS that is independent of RT in patients with hereditary retinoblastoma. (NCCN Soft Tissue Sarcoma, v.2.2024)

The NCCN clinical practice guideline that addresses acute lymphoblastic leukemia (ALL) states that Identification of specific recurrent genetic abnormalities is critical for disease evaluation, optimal risk stratification, and treatment planning. NCCN considers patients with B-cell ALL who have KMT2A (MLL) rearranged molecular alterations as patients with a poor risk, and states that allogenic hematopoietic cell transplant (HCT) for adolescents and young adults with Ph-negative ALL in the first complete remission may be considered for high-risk cases. The NCCN guideline indicates that allogenic HCT should be considered particularly for patients with disease that is minimal/measurable residual disease positive any time after induction; or patients with elevated WBC counts; or patients with B-cell ALL and poor-risk cytogenetics [e.g., hypodiploidy, KMT2A (MLL) rearrangement] at diagnosis as allogeneic HCT in first complete remission improved disease-free survival outcomes. With regard to autologous HCT, NCCN stated that autologous HCT does not result in significant benefit compared with chemotherapy alone in patients with Ph-negative high risk ALL. (NCCN Acute lymphoblastic leukemia, v2.2024)

The NCCN clinical practice guideline addressing acute myeloid leukemia (AML) states that patients with mixed lineage leukemia gene (MLL, also called KMT2A) has been associated with intermediate to poor prognosis and can be characterized by partial tandem duplication in the KMT2A gene (KMT2A-PTD) with KMT2A-PTD being associated with reduced overall survival. (NCCN Acute myeloid leukemia, v3.2024)

In the NCCN guidelines for myeloid/lymphoid neoplasms with eosinophilia and tyrosine kinase gene fusions, while not giving guidelines, NCCN states that mutations detected by NGS may provide a means to identify primary (clonal/ neoplastic) eosinophilia from secondary (reactive) eosinophilia. Mutations described include TET2. A recent survey of 61 patients with WHO-defined myeloid/lymphoid neoplasms associated with eosinophilia and harboring PDGFRA, PDGFRB, FGFR1, or PCM1:JAK2 identified that 14 patients (23%) had at least one mutation, which included TET2. (NCCN Myeloid/Lymphoid Neoplasms, v2.2024)

In the NCCN guidelines for acute myeloid leukemia subsection regarding adult blastic plasmacytoid dendritic cell neoplasm evaluation and work up, NCCN recommends molecular analysis of the most common aberrations which include TET2. (NCCN Acute Myeloid Leukemia, v3.2024)

The NCCN guidelines addressing acute lymphoblastic leukemia (ALL) lists JAK-STAT (CRLF2r,o EPORr, JAK1/2/3r, TYK2r, mutations of SH2B3, IL7R, JAK1/2/3), as cytogenetic and molecular alterations with poor risk in the Cytogenetic and Molecular Prognosis Risk Stratification for B-cell ALL (B-ALL) table with a rating of 2A. (NCCN, Acute Lymphoblastic Leukemia, v2.2024)

The NCCN guideline for T-cell lymphomas states Next-Generation Sequencing (NGS) studies have identified a high frequency of mutations in genes in the JAK-STAT pathway that could contribute to the pathogenesis of T-PLL and JAK3 mutations have been associated with a significant negative impact on overall survival. (NCCN T-Cell Lymphomas, v4. 2024)

The NCCN clinical practice guidelines for T-Cell Lymphomas, genetic testing for STAT5B mutation is recommended for hepatosplenic T-Cell Lymphoma and T-Cell large granular lymphocytic leukemia. (NCCN T-Cell Lymphomas, v4. 2024)

In the most recent clinical practice guidelines for non-small cell lung cancer, NCCN states that progression-free survival (PFS) is longer with use of EGFR TKI monotherapy in patients with the common EGFR mutations when compared with cytotoxic systemic therapy and makes the following recommendations:

- Testing for EGFR mutations, including common and uncommon mutations, in eligible patients with metastatic NSCLC.
- Molecular testing for EGFR mutations is also recommended for eligible patients with respectable stage IB to IIIA and stage IIIB (only T3,N2) NSCLC to determine whether adjuvant therapy with osimertinib is an option.
- Testing for EGFR mutations and other biomarkers in patients with metastatic nonsquamous NSCLC or NSCLC NOS based on data showing the efficacy of afatinib, dacomitinib, erlotinib, gefitinib, or osimertinib and on FDA approvals. (NCCN Non-Small Cell Lung Cancer, v7.2024)

In the NCCN Biomarkers Compendium, molecular testing for KRAS mutations is recommended in the oncology guidelines for Colorectal Cancer Screening with Category 2A recommendations. (NCCN, 2024)

NCCN breast cancer guidelines indicate that “gene expression assays provide prognostic and therapy-predictive information that complements tumor (T), node (N), distant metastasis (M) and biomarker information. Use of these assays is not required for staging.” NCCN categorizes the MammaPrint for consideration of adjuvant systemic therapy in individuals with invasive breast cancer as follows:

Assay	Predictive	Prognostic	NCCN Category of Preference	NCCN Category of Evidence and Consensus
MammaPrint for pN0 and pN1 (1 – 3 positive nodes)	Not determined	Yes	Other	1

(NCCN, Breast Cancer, v4.2024)

The NCCN Guidelines support ancillary diagnostic testing (including with GEPs) to better classify melanocytic neoplasms of uncertain diagnostic potential. As noted in the guidelines, "Ancillary tests to differentiate benign from malignant melanocytic neoplasms include immunohistochemistry (IHC) and molecular testing via comprehensive genomic hybridization (CGH), fluorescence in situ hybridization (FISH), gene expression profiling (GEP), single-nucleotide polymorphism (SNP) array, and Next-Generation Sequencing (NGS). These tests may facilitate a more definitive diagnosis and guide therapy in cases that are diagnostically uncertain or controversial by histopathology.” The guidelines further recommend that ancillary tests should be used as adjuncts to clinical and expert dermatopathologic examination and consultation and therefore need to be interpreted within the context of these findings. (NCCN Melanoma: Cutaneous, v3.2024)

The NCCN Guidelines for thyroid carcinoma support the management for nodules classified as Bethesda III-IV. For Fine needle aspiration (FNA) results showing atypia of undetermined significance (AUS) (Bethesda III) and there is not a high clinical and/or radiographic suspicion of malignancy, NCCN recommends considering repeat FNA, molecular diagnostics, diagnostic lobectomy if Bethesda III on two or more occasions, and nodule surveillance as recommended by the American Thyroid Association (ATA) or Thyroid Imaging Reporting & Data System (TI-RADS). For FNA results does not show follicular or oncocytic neoplasm (Bethesda IV), NCCN recommends considering diagnostic lobectomy, molecular diagnostics, nodule surveillance if low risk or patient preference as recommended by the ATA or TI-RADS. Molecular diagnostic results and treatment recommendations for AUS (Bethesda III) or follicular neoplasm (Bethesda IV) are

categorized as either molecular diagnostics indicate benign lesion, molecular diagnostics not informative or with insufficient or degraded sample, or molecular diagnostics suggestive of malignancy. (NCCN Thyroid Carcinoma, v4.2024)

The NCCN Guidelines address risk stratification of local cutaneous squamous cell carcinoma (cSCC) based on risk factors for local recurrence, metastases, or death. After biopsy, a risk assessment of the primary tumor should be performed as referenced in the "Stratification to Determine Treatment Options and Follow-up for Local CSCC Based on Risk Factors for Local Recurrence, Metastases, or Death from Disease" table. Risk category assignment should be based on the highest risk factor present. The high-risk group has elevated risk of local recurrence while the very-high-risk group has elevated risks of local recurrence and metastasis. (NCCN Squamous Cell Skin Cancer, v1.2024)

National Institutes of Health (NIH), National Cancer Institute (NCI)

More than 30% of all human cancers (including nearly all pancreatic cancers and 45 percent of colorectal cancers) are caused by mutations in the RAS family of genes of which KRAS is one. These cancers are known to be resistant to chemotherapies and due to the structure of mutant RAS proteins, effective therapies are difficult to target. In 2013, NCI established the RAS Initiative to develop ways to understand and target cancers driven by RAS oncogene mutations, innovative approaches for attacking the proteins encoded by mutant forms of RAS genes and to ultimately create effective, new therapies for RAS-related cancers.

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Policy History/Revision Information

Date	Summary of Changes
02/01/2025	**Applicable Codes** ***Non-Covered Diagnosis Codes*** • Added Z59.71 and Z59.72 • Added notation to indicate Z59.7 was “deleted Sep. 30, 2024” • Removed Z11.52 **Supporting Information** • Archived previous policy version MMP383.21

Instructions for Use

The Medicare Advantage Policy documents are generally used to support UnitedHealthcare coverage decisions. It is expected providers retain or have access to appropriate documentation when requested to support coverage. This document may be used as a guide to help determine applicable:

- Medical necessity coverage guidelines; including documentation requirements, and/or
- Medicare coding or billing requirements.

Medicare Advantage Policies are applicable to UnitedHealthcare Medicare Advantage Plans offered by UnitedHealthcare and its affiliates. This Policy is provided for informational purposes and does not constitute medical advice. It is intended to serve only as a general reference and is not intended to address every aspect of a clinical situation. Physicians and patients should not rely on this information in making health care decisions. Physicians and patients must exercise their independent clinical discretion and judgment in determining care. Treating physicians and healthcare providers are solely responsible for determining what care to provide to their patients. Members should always consult their physician before making any decisions about medical care.

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 member specific benefit plan document identifies which services are covered, which are excluded, and which are subject to limitations. In the event of a conflict, the member specific benefit plan document supersedes this policy. For more information on a specific member's benefit coverage, please call the customer service number on the back of the member ID card or refer to the Administrative Guide.

Medicare Advantage Policies are developed as needed, are regularly reviewed, and updated, and are subject to change. They represent a portion of the resources used to support UnitedHealthcare coverage decision making. UnitedHealthcare may modify these Policies at any time by publishing a new version on this website. Medicare source materials used to develop these policies may include, but are not limited to, CMS statutes, regulations, National Coverage Determinations (NCDs), Local Coverage Determinations (LCDs), and manuals. This document is not a replacement for the Medicare source materials that outline Medicare coverage requirements. The information presented in this Policy is believed to be accurate and current as of the date of publication. Where there is a conflict between this document and Medicare source materials, the Medicare source materials apply. Medicare Advantage Policies are the property of UnitedHealthcare. Unauthorized copying, use, and distribution of this information are strictly prohibited.

UnitedHealthcare follows Medicare coverage guidelines found in statutes, regulations, NCDs, and LCDs to determine coverage. The clinical coverage criteria governing certain items or services referenced in this Medical Policy have not

been fully established in applicable Medicare guidelines because there is an absence of any applicable Medicare statutes, regulations, NCDs, or LCDs setting forth coverage criteria and/or the applicable NCDs or LCDs include flexibility that explicitly allows for coverage in circumstances beyond the specific indications that are listed in an NCD or LCD. As a result, in these circumstances, UnitedHealthcare applies internal coverage criteria as referenced in this Medical Policy. The internal coverage criteria in this Medical Policy was developed through an evaluation of the current relevant clinical evidence in acceptable clinical literature and/or widely used treatment guidelines. UnitedHealthcare evaluated the evidence to determine whether it was of sufficient quality to support a finding that the items or services discussed in the policy might, under certain circumstances, be reasonable and necessary for the diagnosis or treatment of illness or injury or to improve the functioning of a malformed body member.

Providers are responsible for submission of accurate claims. Medicare Advantage Policies are intended to ensure that coverage decisions are made accurately. UnitedHealthcare Medicare Advantage Policies use Current Procedural Terminology (CPT®), Centers for Medicare and Medicaid Services (CMS), or other coding guidelines. References to CPT® or other sources are for definitional purposes only and do not imply any right to reimbursement or guarantee claims payment.

For members in UnitedHealthcare Medicare Advantage plans where a delegate manages utilization management and prior authorization requirements, the delegate's requirements need to be followed.