Molecular Oncology Companion Diagnostic Testing

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➲ Instructions for Use

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Application

This Medical Policy does not apply to the states listed below; refer to the state-specific policy/guideline, if noted:

State	Policy/Guideline
Indiana	None
Kentucky	Molecular Oncology Companion Diagnostic Testing (for Kentucky Only)
Louisiana	Molecular Oncology Companion Diagnostic Testing (for Louisiana Only)
Nebraska	Molecular Oncology Companion Diagnostic Testing (for Nebraska Only)
New Jersey	Molecular Oncology Companion Diagnostic Testing (for New Jersey Only)
New Mexico	Molecular Oncology Companion Diagnostic Testing (for New Mexico Only)
North Carolina	None
Ohio	Molecular Oncology Companion Diagnostic Testing (for Ohio Only)
Pennsylvania	Molecular Oncology Companion Diagnostic Testing (for Pennsylvania Only)
Tennessee	Molecular Oncology Companion Diagnostic Testing (for Tennessee Only)

Coverage Rationale

Companion Diagnostic Tests using Comprehensive Genomic Profiling (CGP) are considered proven and medically necessary when used for the appropriate oncology indication when all of the following criteria are met:

- Indication has a corresponding diagnostic test and biomarker on the U.S. Food and Drug Administration (FDA) List of Cleared or Approved Companion Diagnostic Devices
- No Comprehensive Genomic Profiling (CGP) has been performed previously for this tumor type and stage
- If testing is done via Liquid Biopsy [e.g., FoundationOne® Liquid CDx (CPT code 0239U) or Guardant360® CDx (CPT code 0242U)], one of the following criteria must be met:
  - The individual undergoing testing is not medically fit for invasive biopsy; or
  - Tumor tissue testing is not feasible; or
  - Circulating tumor DNA (ctDNA) testing is the proven method for detection of the specific biomarker (e.g., *ESR1* resistance mutations)

Companion Diagnostic Tests using Comprehensive Genomic Profiling (CGP) which do not meet the above requirements are considered unproven and not medically necessary.

Note: For anaplastic thyroid cancer, refer to the Medical Policy titled Molecular Oncology Testing for Solid Tumor Cancer Diagnosis, Prognosis, and Treatment Decisions. For acute myeloid leukemia, refer to the Medical Policy titled Molecular Oncology Testing for Hematologic Cancer Diagnosis, Prognosis, and Treatment Decisions.

Definitions

Advanced Cancer: Cancer that is unlikely to be cured or controlled with treatment. This may also be called end-stage cancer or terminal cancer [National Cancer Institute (NCI), Advanced Cancer, 2023].

Companion Diagnostic Test: A test that provides important information for the safe and effective use of a corresponding therapeutic drug [U.S. Food and Drug Administration (FDA), 2023].

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

Liquid Biopsy: Testing performed on a sample of bodily fluid to identify cancer cells from a tumor or pieces of DNA, RNA or other molecules that have been released from tumor cells and are circulating in an individual's body fluids. Liquid Biopsy may be used for early detection of cancer, to help identify effective treatments or to monitor for return of cancer (NCI, Liquid Biopsy, 2023).

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

Applicable Codes

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

CPT Code	Description
0022U	Targeted genomic sequence analysis panel, non-small cell lung neoplasia, DNA and RNA analysis, 23 genes, interrogation for sequence variants and rearrangements, reported as presence or absence of variants and associated therapy(ies) to consider
0037U	Targeted genomic sequence analysis, solid organ neoplasm, DNA analysis of 324 genes, interrogation for sequence variants, gene copy number amplifications, gene rearrangements, microsatellite instability and tumor mutational burden
0179U	Oncology (non-small cell lung cancer), cell-free DNA, targeted sequence analysis of 23 genes (single nucleotide variations, insertions and deletions, fusions without prior knowledge of partner/breakpoint, copy number variations), with report of significant mutation(s)
0239U	Targeted genomic sequence analysis panel, solid organ neoplasm, cell-free DNA, analysis of 311 or more genes, interrogation for sequence variants, including substitutions, insertions, deletions, select rearrangements, and copy number variations
0242U	Targeted genomic sequence analysis panel, solid organ neoplasm, cell-free circulating DNA analysis of 55-74 genes, interrogation for sequence variants, gene copy number amplifications, and gene rearrangements
0473U	Oncology (solid tumor), next-generation sequencing (NGS) of DNA from formalin-fixed paraffin-embedded (FFPE) tissue with comparative sequence analysis from a matched normal specimen (blood or saliva), 648 genes, interrogation for sequence variants, insertion and deletion alterations, copy number variants, rearrangements, microsatellite instability, and tumor-mutation burden
81445	Solid organ neoplasm, genomic sequence analysis panel, 5-50 genes, interrogation for sequence variants and copy number variants or rearrangements, if performed; DNA analysis or combined DNA and RNA analysis

CPT Code	Description
81449	Solid organ neoplasm, genomic sequence analysis panel, 5-50 genes, interrogation for sequence variants and copy number variants or rearrangements, if performed; RNA analysis
81450	Hematolymphoid neoplasm or disorder, genomic sequence analysis panel, 5-50 genes, interrogation for sequence variants, and copy number variants or rearrangements, or isoform expression or mRNA expression levels, if performed; DNA analysis or combined DNA and RNA analysis
81451	Hematolymphoid neoplasm or disorder, genomic sequence analysis panel, 5-50 genes, interrogation for sequence variants, and copy number variants or rearrangements, or isoform expression or mRNA expression levels, if performed; RNA analysis
81455	Solid organ or hematolymphoid neoplasm or disorder, 51 or greater genes, genomic sequence analysis panel, interrogation for sequence variants and copy number variants or rearrangements, or isoform expression or mRNA expression levels, if performed; DNA analysis or combined DNA and RNA analysis
81456	Solid organ or hematolymphoid neoplasm or disorder, 51 or greater genes, genomic sequence analysis panel, interrogation for sequence variants and copy number variants or rearrangements, or isoform expression or mRNA expression levels, if performed; RNA analysis
81479	Unlisted molecular pathology procedure
81599	Unlisted multianalyte assay with algorithmic analysis

Description of Services

Companion Diagnostic (CDx) Testing refers to the use of in vitro analysis of biological samples to provide information that is necessary for the use of a therapeutic drug (Valla et al., 2021). CDx Testing looks for specific biomarkers that are found in an individual with cancer. The presence or absence of a biomarker or biomarkers can guide treatment decisions and/or determine tumor response to the treatment (NCI, Biomarker Testing for Cancer Treatment, 2021). The first predictive biomarker that was linked to development of a drug was the HER2 protein. The result of this pairing was the 1998 FDA approval of trastuzumab (Herceptin) and the HER2 immunohistochemical assay (HercepTest) for identification and treatment of metastatic HER2-positive breast cancer. Since then, rapid growth in the development of predictive biomarker assays which are associated with specific pharmaceutical treatment agents has occurred (Valla et al., 2021).

This policy addresses CDx performed using Comprehensive Genomic Profiling (CGP) to identify individuals who:
- are most likely to benefit from a particular product
- May be at an increased risk for significant side effects if treated with a particular product
- Require monitoring for response to treatment with a particular product for the purposes of adjusting therapy to improve outcomes

The intended use of a CDx test is described in the labeling of the test, which indicates either a specific therapeutic product or a specific group of oncology therapeutic products. This information is included in the labeling of the therapeutic product as well, including any generic or biosimilar equivalents. It is critical that the diagnostic test is accurate; otherwise, the treatment decision made as a result of the test outcome will not be accurate. In this policy, the U.S. Food and Drug Administration (FDA) List of Cleared or Approved Companion Diagnostic Devices is leveraged for accurate and up-to-date information regarding the use of CDx. (U.S. FDA, 2023)

Clinical Evidence

Comprehensive Genomic Profiling (CGP)

Solid Tumor Tissue Testing

In a 2022 bioinformatic analysis and meta-analysis, Cao et al. investigated the predictive efficacy of tumor mutational burden (TMB) testing when used as a biomarker for individuals with cancer that received treatment with immune checkpoint inhibitors (ICI). Outcomes included objective response rate (ORR), durable clinical benefit (DCB), overall survival (OS) and progress-free survival (PFS) in individuals with high TMB as compared to those with low TMB. Simple nucleotide variation (SNV) information from The Cancer Genome Atlas (TCGA) including 33 major cancer types was used for the non-ICI group; OS was compared between individuals with high TMB in the non-ICI group and the meta-analysis results. A total of 41 studies including 7,713 participants met inclusion criteria and were part of the evaluation. Individuals

with high TMB results had a better ORR (RR = 2.73; 95% CI: 2.31–3.22; p = 0.043) and DCB (RR = 1.93; 95% CI: 1.64–2.28; p = 0.356) as well as a significantly higher OS (HR = 0.24; 95% CI: 0.21–0.28; p < 0.001) and PFS (HR = 0.38; 95% CI: 0.34–0.42; p < 0.001) when compared with individuals with low TMB results. In addition, the study found that immunotherapy may improve OS in certain cancer types with high TMB and more positive prognosis when compared with non-ICI therapy group. These cancer types included colorectal cancer, lung cancer, melanoma, gastric cancer, and pan-cancer. Based on the results of this analysis, the researchers concluded that TMB shows promise for use as a biomarker for immunotherapy treatment. They recommend establishing a standard for TMB assessment including cut-off values, to improve management of various cancer types.

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

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

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

Marcus et al. (2021) summarized the FDA approval of pembrolizumab for treatment of adults and children with unresectable or metastatic TMB-high (defined as ≥ 10 mut/Mb) solid tumors. The approval specifies that TMB must be

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

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

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

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

FoundationOne CDx

FoundationOne CDx (F1CDx) is an FDA-approved panel that is used as a companion diagnostic test to help identify individuals who might benefit from treatment in accordance with FDA product labeling for 28 unique drug therapies. NGS-based CGP methodology is used in F1CDx to analyze 324 genes associated with cancer in solid tumor tissue. Known and likely pathogenic short variants, copy number alterations and select rearrangements as well as biomarkers including tumor mutational burden (TMB) and microsatellite instability (MSI), and in ovarian cancer, genomic loss of heterozygosity

(gLOH) are reported with F1CDx. Included in a 2022 clinical and analytical validation were multiple comprehensive evaluations of F1CDx including limit of detection, limit of blank, precision, and orthogonal concordance for short variants, copy number alterations, genomic rearrangements and select biomarkers. This assay validation includes over 30,000 test results added to the growing body of evidence supporting clinical utility of F1CDx for matching individuals with solid tumors to targeted treatments based on their tumor's genomic variations and biomarkers. (Milbury et al., 2022)

In 2023, Pinet et al. (included in the 2022 Hayes report on FoundationOne CDx) studied the implications of FoundationOne CDx use for individuals with a poor cancer prognosis and few treatment options or in individuals whose cancer is progressing despite at least one course of standard treatment, to assist with therapeutic decision-making including clinical trial participation, early access to certain treatments, or compassionate care. Genomic testing was performed on samples from 150 participants. The testing revealed 2,419 genetic variations. The median variations per tumor was 11. Most frequently identified variants were in TP53, TERT, PI3KCA, CDKN2A/B, KRAS, CCDN1, FGF19, FGF3, and SMAD4. Median TMB was found to be three mutations/Mb (available for 143 individuals). Thirteen individuals (8.6% of the total 150 individuals with known or likely pathogenic variations) underwent matched targeted treatment based on their FoundationOne CDx test results. An additional 60 participants received recommendations from the Molecular Tumor Board (69 individuals were submitted for recommendation). The study found that the therapy provided based on genotype direction did not affect overall survival [13 months vs. 14 months; p = 0.95; hazard ratio = 1.04 (95% confidence interval, 0.48-2.26)]. Based on these results, the authors concluded that a treatment center including a multidisciplinary Molecular Tumor Board and a system for NGS screening can attain outcomes comparable to larger treatment centers for decisions such as including individuals with advanced cancers in clinical trials.

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

Hayes addressed the use of FoundationOne CDx for use as a broad molecular profiling tool in a 2022 Molecular Test Assessment. The evidence base for this indication consisted of three clinical utility studies which reported no difference in outcomes between treatment directed by FoundationOne CDx results and treatment not directed by use of FoundationOne CDx. As such, the evidence was determined to be insufficient for this indication. The Hayes report did not assess the use of FoundationOne CDx for the primary purpose of evaluating predetermined biomarkers that are associated with at least one FDA-approved therapy for the individual's specific cancer type, nor did it address clinical or analytical validity, which would require focused review of individual biomarkers [Hayes, FoundationOne CDx (Foundation Medicine Inc.) for the Intended Use as a Broad Molecular Profiling Tool, 2022 updated 2023].

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

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

implementation of genomically guided therapy were clinical status of the patient and drug access. Early and serial sequencing in the clinical course and expanded access to genomically guided early-phase clinical trials and targeted agents may increase clinical application.

Frampton and colleagues (2013) conducted an analytical and clinical validation study to evaluate massively parallel DNA sequencing using the FoundationOne assay to characterize base substitutions, indels, copy number alterations, and selected fusions across 287 cancer-related genes from routine formalin-fixed and paraffin-embedded (FFPE) clinical specimens. The authors implemented a validation strategy with reference samples of pooled cell lines that modeled key drivers of test accuracy, including mutant allele frequency, indel length and amplitude of copy change. Test sensitivity achieved was 95% to 99% across alteration types, with high specificity [positive predictive value (PPV) > 99%]. The authors confirmed accuracy using 249 FFPE cancer specimens characterized by established assays. Application of the test to 2,221 clinical cases revealed clinically actionable alterations in 76% of tumors, three times the number of actionable alterations detected by current diagnostic tests. This study did not evaluate the clinical utility of such findings in improving care and outcome of patients by tailoring treatments or predicting response to treatment. Hence, it is important to note that the clinical utility of genomic profiling using massively parallel DNA sequencing remains unknown. In addition, study authors colleagues did not categorize the data regarding sensitivity, specificity, and positive predictive value (PPV) by cancer type.

FoundationOne Heme

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

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

Guardant360 TissueNext™ (Guardant Health, Inc.)

Guardant360 TissueNext is a CGP panel test performed on tumor tissue that is meant to influence treatment decisions in individuals with advanced cancer. A recent Hayes Precision Medicine Research Brief uncovered no published abstracts which assessed the clinical validity or utility of the Guardant360 TissueNext test and concluded that at this time, there is insufficient peer-reviewed published literature to support the use of Guardant360 TissueNext [Hayes, Guardant360 TissueNext (Guardant Health Inc.), 2023].

MI Profile™ and MI Tumor Seek™ (Caris Life Sciences)

In 2022 (updated 2023), Hayes published a Molecular Test Assessment on the MI Profile (Caris Life Sciences) for the proposed use as a broad molecular profiling tool to detect tumor biomarkers and allocate matched therapy specific to those biomarkers for individuals with solid tumors. The MI Profile performs multiplatform solid tumor biomarker analysis by using DNA (NGS-based WES), RNA (NGS-based whole transcriptome sequencing) and proteins from solid tumor tissue samples to report on biomarker variation results, therapeutic agents associated with biomarker results and finally, applicable open clinical trials the individual may be eligible for, to assist oncologists with treatment decisions. The review uncovered no peer-reviewed studies meeting the inclusion criteria for evaluation of clinical utility; as such, overall quality of evidence was not rated and at this time and Hayes concluded that there is insufficient data to support clinical utility of the MI Profile for use as a broad molecular profiling tool at this time. The Hayes report did not address the use of this test for the primary purpose of testing limited biomarkers that have one or more associated FDA-approved therapies for the specific cancer types, or the analytical or clinical validity of the test [Hayes, MI Profile (Caris Life Sciences) for the Intended Use as a Broad Molecular Profiling Tool, 2022].

The MI Tumor Seek is a tumor profiling platform which evaluates DNA mutations, copy number alterations, insertions/deletions, genomic signatures such MSI and TMB and RNA whole transcriptome sequencing with a goal of detecting tumor biomarkers that may help providers identify a personalized cancer treatments. MI Tumor Seek is NGS-based and uses WES analysis. In a 2018 publication by Vanderwalde et al., the ability of the Caris NGS platform to detect MSI was assessed. The association of MSI with TMB and PD-L2 were also examined. The researchers analyzed a total of 2,189 individuals with 26 different types of cancer and compared the mismatch repair status found with the NGS platform with PCR fragment analysis for the same group. When compared with MIS by PCR fragment, the MSI by NGS had a sensitivity of 95.8% (95% CI, 92.24-98.08), specificity of 99.4% (95% CI, 98.94-99.69), positive predictive value of 94.5% (95% CI, 90.62-97.14), and negative predictive value of 99.2% (95% CI, 98.75-99.57). Elevated MSI was detected in 23 of the 26 cancers. These results indicate that Caris's NGS platform was able to ascertain MSI status regardless of cancer type. This 2018 study, however, may not be identical to the currently marketed test and no validity of the test process was assessed. In addition, the study had potential selection bias, as the individuals included had advanced disease and lack of any clear options for therapy. Additional investigation is required to further define relationships between TMB, MSI and PD-L1.

Liquid Biopsy

Liquid biopsy is a non-invasive technique of obtaining bodily fluids, such as blood, urine, cerebrospinal fluid, saliva, and other aspirates, to analyze different types of biomolecules including circulating tumor DNA (ctDNA), circulating tumor cells (CTCs), and exosomes. Research continues to study this technique for non-invasive methods that may assist in therapeutic decisions without traditional biopsy.

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

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

In a 2022 systematic review and meta-analysis, Zhang et al. studied the predictive value of TMB in the blood (bTMB) using studies evaluating bTMB use in ICIs or the efficacy of ICIs compared with chemotherapy. A total of seven trials including 2,610 individuals with NSCLC were included in the systematic review. No significant differences between high and low bTMB groups in the ICI cohort were found with regard to OS (HR = 1.09; 95% CI: 0.62–1.91, p = 0.774) or PFS (HR = 0.73; 95% CI: 0.20–2.65, p = 0.629). In the comparisons of ICI to chemotherapy, ICIs showed improvement in OS (HR = 0.74; 95% CI: 0.59–0.92, p = 0.006), but improvement in PFS and ORR was attributable to a mathematical trend only (PFS: HR = 0.83; 95% CI: 0.63–1.09, p = 0.173; ORR: RR = 0.92, 95% CI: 0.77–1.10, p = 0.372). Participants treated with ICIs in the high bTMB group had greater survival benefits than individuals receiving chemotherapy in terms of OS (HR = 0.63; 95% CI: 0.51–0.76, p < 0.001), PFS (HR = 0.63; 95% CI: 0.52–0.76, p < 0.001), and ORR (RR = 1.86; 95% CI: 1.32–2.62, p < 0.001). In the low TMB group, there was either no change in the outcome or a reversal of the findings in the high bTMB group (OS: HR = 0.89; 95% CI: 0.64–1.24, p = 0.485; PFS: HR =1.21, 95% CI: 0.93–1.58, p = 0.154; ORR: RR = 0.68, 95% CI: 0.54–0.85, p = 0.001). Limitations included the heterogeneity of the studies, the risk of bias, and the retrospective nature of the studies reviewed. The authors concluded that TMB has been shown to be a reliable biomarker for identifying individuals with NSCLC who may benefit from ICI. The role of bTMB remains limited at this time, and more prospective data are needed.

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

A Hayes Clinical Utility Evaluation indicates that evidence documenting the ability of liquid biopsy testing to identify early-stage colorectal cancer and high-risk adenoma accurately in an unselected, prospective population is insufficient to support conclusions regarding clinical utility at this time. Per the Hayes report, evidence for other types of liquid biopsy screening tests for CRC are lacking as well (Hayes, Liquid Biopsy Tests for Colorectal Cancer Screening, 2020, updated 2023).

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

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

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

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

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

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

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

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

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

FoundationOne Liquid CDx

FoundationOne Liquid CDx (Foundation Medicine, Cambridge, MA) is an FDA-approved test that can detect gene variations (> 300 genes tested) in circulating cfDNA that has been isolated from whole blood plasma samples (also referred to as “liquid biopsy”). Results can help providers identify individuals that might benefit from certain cancer drugs.

Bayle et al. (2023) reported results from a prospective study which explored the use of CMP of ctDNA in individuals with advanced solid tumor cancers. The FoundationOne Liquid CDx was used to obtain genomic evaluation on 1,772 individuals with metastatic solid tumors. The results of 1,658 were used in the analysis. Actionable targets were identified using the ESMO Scale for Clinical Actionability of Molecular Targets (ESCAT). In 1,059 participants, at least one actionable target was identified (64%); 1,825 actionable variations, total, were found. Results were reviewed by a multidisciplinary tumor board and a matched therapy was advised for 56% (597) individuals. Ultimately, 122 individuals underwent treatment; data was available for 107 of those. Median progression-free survival was 4.7 months (95% confidence interval 2.7-6.7 months), and median overall survival was 8.3 months (95% confidence interval 4.7-11.9 months). The researchers concluded that ctDNA sequencing using a large CMP panel can be efficiently used to match individuals with advanced solid tumor cancers to targeted treatments.

Caputo et al. (2022) used the FoundationOne Liquid Analysis [either FoundationOne Liquid (70 genes) or FoundationOne CDx (324 genes)] to evaluate clinical impact and viability of these tests across different tumor types. In all, 398 samples from various tumor types were evaluated with an overall success rate of 92% (97% success rate in FoundationOne Liquid

CDx individually). The most common molecular alterations were TP53 (74), APC (40), DNMT3A (39) and KRAS (23). Overall clinical impact of FoundationOne Liquid Analysis use compared to standard diagnostic testing was 64.7% vs. 22.1% [risk ratio (RR) = 2.94; p < 0.001] and potential clinical impact was 58.6% compared to 11.0% (RR = 5.32; p < 0.001). Also noted is that FoundationOne Liquid Analysis detected actionable alterations that offered an unexpected therapeutic choice. The authors assert that NGS using FoundationOne Liquid Analysis is a helpful assay to guide treatment decisions in oncology, but comment that more study is needed in terms of selection criteria for affected individuals to avoid over-diagnosis.

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

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

Guardant360® CDx

Guardant360 CDx (Guardant Health, Redwood City, CA) is an FDA-approved liquid biopsy for advanced solid tumors, intended to be used as a companion diagnostic to identify patients with non-small cell lung cancer (NSCLC) who might benefit from targeted therapies. This test uses circulating cell-free DNA (cfDNA) from the plasma of peripheral whole blood and high throughput hybridization-based capture technology to detect single nucleotide variants (SNVs), insertions and deletions in 55 genes, fusions in 4 genes and copy number amplifications (CNAs) in 2 genes.

In an updated Molecular Test Assessment, Hayes explored the evidence on Guardant360 CDx test and evaluated its effect on the survival of individuals diagnosed with solid tumor cancers. The previous version of the report focused on test performance and potential changes in the management of individuals with cancer. The updated report focuses on clinical outcomes. A very low-quality body of evidence was found; studies found were of small size/number of events, they contained insufficient records regarding the statistical plan, studies were retrospective, and the evidence regarding whether treatment decisions were actually based on the outcomes of Guardant360 CDx testing was unclear. Hayes indicates that more studies with larger populations and longer follow-up are needed and recommend studies comparing targeted multigene panels with Guardant 360 CDx for specific cancer types (Hayes, Guardant360 CDx (Guardant Health, Inc., 2023).

Olsen et al. (2022, included in Hayes Comprehensive Molecular Profiling of Circulating Solid Tumor DNA for the Intended Use as a Broad Molecular Profiling Tool to Aid Treatment Selection, 2022 and Hayes Guardant360 CDx) evaluated data from 3,084 individuals with advanced NSCLC who had been registered in a real-world healthcare claims database and had undergone NGS-based circulating tumor DNA (ctDNA) testing with Guardant360 after first-line treatment. In 89.9% of the samples, ctDNA was detected and 41.9% of those samples showed actionable variations (most commonly EGFR –

29.7%).Of individuals previously treated with non-targeted drugs, actionable alterations were found in 26.7% of individuals and emerging and potentially targetable mutations were found in 40.1%. In patients whose ctDNA testing showed qualifying alterations, time to discontinuation of therapy and overall survival were longer in individuals who received matched second-line treatment versus unmatched second-line treatment. The authors concluded that use of blood-based NGS assays before second-line treatment helps to inform treatment-making decisions that may improve clinical outcomes in individuals with advanced NSCLC in a real-world practice situation. Of note, this study was limited to biomarker testing using only the Guardant Health testing platform and Guardant Health funded this study.

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

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

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

McCoach et al. (2018) evaluated patients with advanced NSCLC and with tumors that carried ALK gene fusions. The researchers sought to analyze the cfDNA to find a non-invasive way to identify these gene fusions. The study used the Guardant360 database of NSCLC cases to identity patients. Eighty-eight patients with 96 plasma-detected ALK fusions

were determined. The fusion partners identified included EML4 (85.4%), STRN (6%), and KCNQ, KLC1, KIF5B, PPM1B, and TGF (totaling 8.3%). The study concluded that in this cohort, cfDNA was acceptable at detecting targetable alterations.

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

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

Clinical Practice Guidelines

American Society of Clinical Oncology (ASCO)

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

- Individuals who have been diagnosed with advanced or metastatic cancer and adequate performance status should be tested with genomic sequencing when:
  - Genomic biomarker-associated therapies exist which have been approved by regulatory agencies for the individual's cancer
  - Treatment for which there are specific biomarker-based contraindications or exclusions exist (strength of recommendation: strong)
- Multigene panel tests should be performed when individual has metastatic or advanced solid tumor and is eligible for genomic biomarker-linked, approved therapy (strength of recommendation: moderate)
- Multigene panel tests should be performed when individual has more than one genomic biomarker associated with an approved therapy (strength of recommendation: strong)
- Testing used to inform clinical care must be done in an appropriately certified laboratory (strength of recommendation: strong)
- Clinical decision making should include:
  - Known or predicted impact of genomic alteration on protein expression/function
  - Clinical data on efficacy of targeting the genomic alteration with a specific treatment agent (strength of recommendation: strong)
- Individuals with advanced or metastatic solid tumors should undergo germline testing for genetic alterations that have been linked to approved therapies under consideration. This should not be limited by clinical criteria for familial risk or family history reports. In addition, individuals with pathogenic or likely pathogenic (P/LP) variations should be referred for genetic counseling (strength of recommendation: strong)
- Evaluation of mismatch repair deficiency status (dMMR) should be performed for individuals with advanced or metastatic solid tumors who are under consideration for use of immunotherapy (strength of recommendation: strong)
- Testing with either large multigene panels including validated TMB testing or whole exome analysis should be performed when TMB may influence decision-making regarding use of immunotherapy (strength of recommendation: strong)
- Individuals with advanced or metastatic solid tumors should undergo fusion testing if there are fusion-targeted therapies approved for their specific disease (strength of recommendation: strong)
- In individuals with advanced or metastatic solid tumors who may be considered for TRK-inhibitor therapy, NTRK fusion testing should be performed (strength of recommendation: strong)
- Individuals with advanced or metastatic solid tumors may be tested for other fusions if no oncogenic driver alterations have been identified on large panel DNA sequencing (strength of recommendation: moderate)
- MET exon 14 skipping testing is recommended for individuals diagnosed with any type of non-small-cell lung cancer (strength of recommendation: strong)

- In individuals with advanced or metastatic solid tumors, genomic testing should be considered in order to determine whether individuals is an appropriate candidate for tumor-agnostic therapies without genomic biomarker-linked therapies (strength of recommendation: moderate)
- When no genomic biomarker-linked targeted therapies exist for potentially actionable genomic alterations, individual participation in clinical trials is encouraged (after considering efficacy of available standard-of-care treatments) (strength of recommendation: strong)
- The use of off-label and off-study biomarker-linked treatments which have been approved for other diseases is not recommended when clinical trial participation is an option or when there is no clinical evidence of meaningful efficacy (strength of recommendation: strong)

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

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

The European Society for Medical Oncology (ESMO)

ESMO’s Precision Medicine workgroup recently made recommendations regarding the use of ctDNA for individuals with cancer (Pascual et al., 2022). The workgroup convened to review analytical and clinical validity and utility of ctDNA assays and found that ctDNA assays with sufficient sensitivity are useful for detecting actionable variations, which can help with decision-making for targeted therapy in individuals with advanced cancers. These assays may be used routinely in clinical practice, as long as the assay limitations are considered. Although tissue-based testing is the desired method in most individuals, ctDNA tests can also be used routinely when speed of results is critical or when tissue biopsies are not feasible. Substantial evidence exists to support the clinical validity of identifying molecular residual disease/molecular relapse in individuals who have been treated for early-stage cancers in terms of predicting future relapse for many cancer types. However, no clinical utility was found for molecular residual disease/molecular relapse detection in routine practice since there is no evidence to support clinical utility for the direction of treatment. The group also made recommendations for future development of ctDNA assays, ongoing research, and recommendations for the reporting of test results.

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

International Association for the Study of Lung Cancer (IASLC)

In a 2021 Consensus Statement from the IASLC, Rolfo et al. acknowledge the dramatic advances in precision medicine on the clinical management of non-small cell lung cancer (NSCLC) and advanced staged cancers overall. The authors note that while the data are most robust for NSCLC, there may well be benefit shown for other cancer types as well,

impacting selection of targeted therapy types, as research progresses. Recommendations from this group now include using a clinically validated NGS platform rather than single gene, PCR-based approaches, considering plasma ctDNA a valid tool for genotyping advanced NSCLC in newly diagnosed patients, and the use of liquid biopsy either as complementary to tissue-based analysis or as the initial approach to biomarker evaluation in oncogene-addicted NSCLC and for monitoring efficacy of therapies. The authors anticipate continued growth of the role of liquid biopsy in both the near and long-term future.

National Institute for Health and Care Excellence (NICE)

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

National Comprehensive Cancer Network (NCCN)

NCCN guidelines for Treatment by Cancer Type address the use of individual tumor markers for specific cancer types as well as the use of multigene panels and molecular profiling. NCCN specifically mentions liquid biopsy (plasma) testing in certain clinical scenarios as well. Studies have demonstrated cell-free tumor DNA generally has very high specificity, but significantly compromised sensitivity, with up to a 30% false-negative rate (NCCN Non-Small Cell Lung Cancer v3.2023). In spite of this, evidence supports use of this testing in certain scenarios. Comments on specific cancer types below:

Ampullary Adenocarcinoma

For ampullary adenocarcinoma, tumor/somatic molecular profiling to identify uncommon mutations is recommended for those individuals with locally advanced/metastatic disease who are candidates for treatment with anti-cancer therapy. Specifically, testing for potentially actionable somatic findings to include fusions (ALK, NRG1, NTRK, ROS1, FGFR2, RET), mutations (BRAF, BRCA1/2, KRAS, PALB2), amplifications (HER2), microsatellite instability (MSI), mismatch repair deficiency (dMMR) or TMB via an FDA-approved and/or validated NGS-based assay. For identifying RNA fusions, RNA sequencing assays are preferred. Testing on tumor tissue is preferable, but cfDNA testing can be considered if tumor tissue testing is not feasible. (NCCN Ampullary Adenocarcinoma, v1.2023).

Bladder Cancer

For bladder cancer, NCCN recommends molecular/genomic testing (in a CLIA-approved laboratory), including FGFR RGQ RT-PCR for *FGFR3* or *FGFR2* genetic alterations, for stages IVA and IVB bladder cancer and consideration of this testing for stage IIIB bladder cancer. Recommendation is for early testing, ideally at diagnosis of advanced bladder cancer, to assist with decision-making. NCCN notes that genetic variations are common in bladder cancer, citing data as the third highest mutated cancer. (NCCN Bladder Cancer, v3.2023).

Bone Cancer

NCCN Bone Cancer guidelines recommends consideration of CGP via validated/FDA-approved assay for individuals with metastatic chondrosarcoma, recurrent chordoma, Ewing sarcoma and metastatic osteosarcoma to identify potential targeted treatment opportunities and encourages impacted individuals to participate in well-designed clinical trials to further advance study. (NCCN Bone Cancer, v3.2023).

Breast Cancer

The NCCN guideline for Breast Cancer indicates that genomic profiling may be performed for use in determining appropriate treatment for breast cancer. In the setting of recurrent unresectable or stage IV breast cancer, testing for biomarkers associated with FDA-approved therapies is recommended. PIK3CA mutations may be assessed with tumor or liquid biopsy to identify candidates for alpelisib plus fulvestrant in individuals with HR-positive/HER2-negative cancer of the breast. PIK3CA mutation testing may be carried out on tumor tissue or ctDNA in peripheral blood (liquid biopsy). If liquid biopsy is negative, tumor tissue testing is recommended. (NCCN Breast Cancer, v4.2023).

Cervical Cancer

For persistent or recurrent cervical cancer, NCCN indicates

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

- If tissue biopsy of metastatic site is not feasible or tissue is not available, CGP via a validated plasma ctDNA assay may be considered. (NCCN Cervical Cancer, v1.2023)

Colorectal Cancer (CRC)

The NCCN guidelines for Colon Cancer and Rectal Cancer indicate that targeted treatment for advanced/metastatic CRC is becoming more common and as such, NCCN has expanded recommendations for biomarker testing. For individuals with metastatic CRC, recommended workup should include determination of tumor gene status for *KRAS/NRAS* and *BRAF* mutations, HER2 amplifications, and MSI/MMR status (if not previously done) either individually or as part of tissue- or blood-based NGS panel test. NGS panels have the advantage of the ability to detect rare and actionable gene alterations such as *NTRK* fusions. The guideline further notes that molecular testing on tissue samples is preferred, but blood-based assays are also an option. Both tissue- and blood-based NGS panels have the ability to pick up rare and actionable mutations and fusions. (NCCN Colon Cancer, v2.2023, NCCN Rectal Cancer, v3.2023).

Histiocytic Neoplasms

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

Gastric and Esophageal/Esophagogastric Junction Cancers

Several target agents have been approved by the FDA for use in gastric, esophageal, and esophagogastric junction cancers. When limited tissue is available for testing, or if a traditional biopsy is not able to be obtained from the individual undergoing evaluation, sequential testing of individual biomarkers or administration of limited molecular panels may exhaust the material available for testing. In these situations, CGP with a validated NGS assay may be used to identify applicable biomarkers (such as HER2 amplification, MSI status, MMR deficiency, TMB, and NTRK gene fusions, RET gene fusions, and BRAF V600E mutations). In solid tumor cancers, genomic alterations can also be identified via ctDNA in the blood. Such testing is becoming more common in individuals with advanced disease; specifically, those individuals who are not able to undergo clinical biopsy for disease surveillance and management. For individuals with metastatic or advanced gastric cancer that cannot undergo traditional biopsy, or in the setting of disease progression monitoring, testing with a validated NGS-based CGP profile using ctDNA may be considered. NCCN cautions that negative results must be interpreted carefully, as this does not necessarily exclude tumor mutations or amplifications. (NCCN Gastric Cancer, v1.2023, NCCN Esophageal and Esophagogastric Junction Cancers, v2.2023).

Melanoma: Cutaneous

Per the NCCN Cutaneous Melanoma guideline, NGS, including various sequencing technologies, allows DNA and RNA sequencing to be performed more quickly and is less costly than Sanger sequencing. Single gene or small multi-gene panels can be used in some cases to test a single gene (e.g., BRAF) or a limited number of genes. Tumor tissue is preferred for molecular testing, however liquid biopsy may be performed if tumor tissue is not available. Broader genomic profiling is recommended, if possible, specifically if the results of testing may help guide future therapeutic decisions or eligibility for lineal trial participation (NCCN Melanoma: Cutaneous, v2.2023).

Non-Small Cell Lung Cancer (NSCLC)

NCCN recommends that, when possible, molecular testing should be performed with a broad, panel-based approach; NGS is most common. Broad molecular profiling is defined as molecular testing that can identify all biomarkers listed in the NSCLC guideline via either single assay or a combination of limited assays. Identification of emerging biomarkers is also desirable and tiered approaches that are based on lower prevalence of co-occurring biomarkers are appropriate for use as well.

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

- Patient is not medically fit for invasive tissue sampling
- In the setting of initial diagnosis, if following pathologic confirmation of a NSCLC diagnosis there is insufficient material for molecular analysis, cell-free/circulating tumor DNA can be used only if follow-up tissue-based analysis is planned for any patient in which an oncogenic driver is not identified
- In the setting of initial diagnosis, if tissue-based testing does not fully assess all recommended biomarkers due to quantity of tissue available or testing methodologies available, consider repeat biopsy and/or cell-free/circulating tumor DNA testing

- In the setting of initial diagnosis, if timely tissue-based testing is not feasible, concurrent cfDNA testing may be helpful for biomarker identification for treatment selection (provided that negative results are understood as per the limitations noted
- In metastatic disease, data suggest that cfDNA testing can be used to identify genes including ALK, BRAF, EGFR, HER2, MET exon 14 skipping, RET, ROS1, and other oncogenic biomarkers that may otherwise not be detected (NCCN Non-Small Cell Lung Cancer, v3.2023)

Ovarian Cancer/Fallopian Tube Cancer/Primary Peritoneal Cancer

NCCN recommends tumor molecular evaluation via validated test(s) in a CLIA-certified laboratory to identify, at a minimum, the potential benefit of targeted therapeutic agents with tumor specific or tumor agnostic benefit. These should include (but are not limited to) BRCA1/2, HRD status, MSI, MMR, TMB, FRα, RET and NTRK, if any prior testing performed did not include these markers. Further testing with more comprehensive panels may be of specific importance in less common ovarian cancers with limited approved options for therapy. Also recommended is molecular testing prior to start of therapy for persistent or recurrent disease if such testing was not already performed. (NCCN Ovarian Cancer/Fallopian Tube Cancer/Primary Peritoneal Cancer, v2.2023).

Pancreatic Adenocarcinoma

NCCN recommends tumor/somatic molecular profiling for individuals with locally advanced/metastatic disease who are candidates for anti-cancer therapy for identification of uncommon mutations. Testing for potentially actionable somatic findings including, but not limited to: fusions (ALK, NRG1, NTRK, ROS1, FGFR2, RET), mutations (BRAF, BRCA1/2, KRAS, PALB2), amplifications (HER2), MSI, dMMR or TMB using an FDA-approved and or validated NGS-based assay. Of note, testing on tumor tissue is preferred; however, cell-free DNA testing can be considered if tumor tissue testing is not feasible. (NCCN Pancreatic Adenocarcinoma, v2.2023).

Prostate Cancer

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

U.S. Food and Drug Administration (FDA)

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

The list of FDA-approved or cleared Companion Diagnostics is available at: List of Cleared or Approved Companion Diagnostic Devices | FDA. (Accessed June 29, 2023)

Laboratories that perform genetic tests are regulated under the Clinical Laboratory Improvement Amendments (CLIA) Act of 1988. More information is available at: https://www.fda.gov/medicaldevices/deviceregulationandguidance/ivdregulatoryassistance/ucm124105.htm. (Accessed June 29, 2023)

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Policy History/Revision Information

Date	Summary of Changes
07/01/2024	**Application** ***New Mexico*** • Added language to indicate this Medical Policy does not apply to the state of **New Mexico**; refer to the state-specific policy version **Applicable Codes** • Updated list of applicable CPT codes to reflect quarterly edits; added 0473U **Supporting Information** • Archived previous policy version CS373.A

Instructions for Use

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

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