FDA Cleared or Approved Companion Diagnostic Testing (for Louisiana Only)
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Instructions for Use

Certain content mandated by Louisiana Department of Health

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Application

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

Coverage Rationale

State-Specific Criteria

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

Genetic Counseling

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

- Obtaining a structured family genetic history; and
- Genetic risk assessment; and
- Counseling of the enrollee and family about diagnosis, prognosis, and treatment

(LA MCO Genetic Counseling and Testing)

Additional Non State-Specific Criteria

Companion Diagnostic Tests are considered proven and medically necessary when the oncology 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 and all of the following criteria are met:

- The Companion Diagnostic Test must align with the drug, FDA-approved indication, and appropriate tissue/specimen in the FDA List of Cleared or Approved Companion Diagnostic Devices; and
- The use of the Companion Diagnostic Test must be consistent with the label for the Companion Diagnostic-associated drug indicated by requesting provider

Repeat Companion Diagnostic Testing on a new tissue or Liquid Biopsy specimen for the purpose of assisting with therapy selection is considered proven and medically necessary up to three times annually when the criteria above for Companion Diagnostic Tests are met and one of the following:

- The individual is experiencing disease recurrence; or
- The individual's cancer has progressed or did not respond to the most recent systemic therapy

Concurrent tissue-based and Liquid Biopsy Companion Diagnostic Testing (ordered within 30 days of each other) is considered proven and medically necessary for the following cancer types when the criteria above for Companion Diagnostic Tests are met:

- Advanced or metastatic (stage IV) breast cancer
- Advanced or metastatic (stage IV) non-small cell lung cancer

Note: If no cancer/diagnostic test match is found on the U.S. Food and Drug Administration (FDA) List of Cleared or Approved Companion Diagnostic Devices, refer to the Medical Policy titled *Molecular Oncology Testing for Solid Tumor Cancer Diagnosis, Prognosis, and Treatment Decisions (for Louisiana Only)* or *Molecular Oncology Testing for Hematologic Cancer Diagnosis, Prognosis, and Treatment Decisions (for Louisiana Only)*.

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, 2024].

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), 2024].

Comprehensive Genomic Profiling (CGP): A type of next-generation sequencing (NGS) test that is able to simultaneously detect all classes of genomic alterations, including cancer biomarkers, across hundreds of genes 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, 2024).

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

CPT Code	Description
*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
0543U	Oncology (solid tumor), next-generation sequencing of DNA from formalin-fixed paraffin-embedded (FFPE) tissue of 517 genes, interrogation for single-nucleotide variants, multi-nucleotide variants, insertions and deletions from DNA, fusions in 24 genes and splice variants in 1 gene from RNA, 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
*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

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

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 FDA-approved or cleared CDx testing performed 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, 2024)

Clinical Evidence

Solid Tumor Tissue Testing

Bogdan et al. (2024) performed a retrospective survey to assess the influence of next-generation sequencing (NGS) panel results from individuals with advanced solid tumors on treatment decision-making. Medical oncologists enrolling participants in the Ontario-wide Cancer Targeted Nucleic Acid Evaluation (OCTANE) study, an ongoing, prospective, multi-facility study including over 4,500 individuals with solid tumor cancers, were surveyed. The goal of the OCTANE study is to use NGS panel testing to develop a registry of tumor tissue and test results described with both clinical and molecular data for use in future research efforts. The survey took place between 2016 and 2021 and the gene panels used were comprised of either 161 or 555 cancer-associated genes (Oncomine Comprehensive Assay V3: ThermoFisher Scientific and Hi5, SureSelect: Agilent, respectively). The primary outcome measured was the rate of treatment change based on gene variation results. A total of 582 surveys were sent; 67.7% (n = 394) were completed and returned. One hundred eighty-eight individuals with a solid tumor cancer had NGS results deemed “actionable” per the treating oncologist and of these, 15.7% (n = 62) were matched to treatment: 37 were enrolled in a clinical trial, 13 received an approved drug, four received off-label therapy, and eight were able to avoid ineffective treatment. Notably, there was not a difference in overall survival (OS) between participants who received matched treatment when compared to those who did not (p = .55, median survival not reached) in this population, however the researchers indicate that the clinical usefulness of NGS panel testing was supported by the outcomes of this study, with 15.7% of participants whose testing revealed actionable results undergoing a change in drug treatment based on those findings. Other factors such as physician, test, and participant characteristics did not show a significant association with treatment changes. The authors further note that rate of treatment changes typically increase as individuals with cancer are followed over time, suggesting that the long-term benefits of NGS panel testing may be underestimated by cross-sectional studies that have relatively short follow-up timeframes. Notable limitations include lack of randomization related to access to matched treatment for individuals with small cell lung cancer and colorectal cancer (CRC) and the use of a survey which may have been impacted by recall bias. In addition, the turnaround time for OCTANE testing results was eight weeks; as such, many participants joined the study while they were undergoing standard treatment and tumor mutational burden (TMB) was not measured with the NGS panels used in this study. Because of these factors, it is possible that the study underestimated the impact of NGS panel testing on treatment decision-making. Ongoing study focused on clinical utility of NGS panel testing in individuals with advanced solid tumor cancers in various health setting is recommended.

To address the lack of existing data focused on patterns of molecular testing in individuals with advanced prostate cancer, Park et al. (2024) directed a retrospective study evaluating NGS testing for affected individuals in the United States. Issues examined included single versus serial NGS, the various disease states when testing was performed (hormone-sensitive versus castration-resistant, metastatic versus nonmetastatic), use of tissue versus circulating tumor DNA (ctDNA), and the rate of actionable findings on each NGS test. The study included information from 1,597 individuals with advanced prostate cancer across 15 institutions using the Prostate Cancer Precision Medicine Multi-Institution Collaborative Effort clinical-genomic database. The researchers defined “actionable” NGS data as results showing somatic variations in homologous recombination repair genes, mismatch repair deficiency (dMMR), microsatellite instability (MSI-high), or a high (≥ 10 mut/MB) TMB. Only nine percent of total study participants (n = 144) underwent serial NGS assessment, defined as two or more NGS tests with testing material collected greater than 60 days apart. Of all NGS tests performed after the first, 82.1% were obtained from ctDNA assays and 76.1% were from individuals with metastatic castration-resistant prostate cancer. Eleven percent of second NGS tests identified new actionable data; 3.5% of these detected a new *BRCA2* alteration or MSI-high. These results led to treatment with targeted therapy in 31.3% of the individuals with new actionable data. Based on their findings, the authors propose that repeat somatic testing using the tissue or blood of individuals with metastatic castration-resistant prostate cancer may well have clinical utility for guiding treatment options.

Molecular assessment for individuals with newly diagnosed metastatic nonsquamous (mNSq) non-small cell lung cancer (NSCLC) is supported by current oncology guidelines, however, the relationship between availability of molecular testing prior to first line treatment and OS is unknown. Aggarwal and colleagues (2023) sought to evaluate this association in a real-world, retrospective cohort study using clinical information of individuals recently diagnosed with mNSq NSCLC. Of 326 participants in the study, 261 (80%) had results of molecular testing available prior to first line treatment and 63 (20%)

did not have available results. Individuals in the group with available testing had significant longer OS when compared to individuals in the group without available results [adjusted hazard ratio, 0.43; 95% confidence interval (CI), 0.30 to 0.62; p < .0001]; median follow up time was 14.2 months. In addition, the adjusted odds of having available results prior to first line treatment was higher with concurrent tissue and plasma testing when compared to tissue testing alone (adjusted odds ratio, 2.06; 95% CI, 1.09 to 3.90; p = .026). This is likely due to the previously reported faster turnaround times for plasma genotyping in comparison with tissue genotyping. These results led the researchers to recommend that renewed focus be placed on the importance of molecular testing prior to first line treatment in individuals with mNSq NSCLC, since the study findings appear to bolster the existing literature demonstrating improved survival rates when guideline-led targeted treatment is initiated after detection of actionable variants. The study also uncovered potential additional survival benefit when molecular test results were available prior to first line treatment. Additional prospective, randomized clinical trials are recommended.

In a 2022 bioinformatic analysis and meta-analysis, Cao et al. investigated the predictive efficacy of TMB testing when used as a biomarker for individuals with cancer who received treatment with immune checkpoint inhibitors (ICI). Outcomes included objective response rate (ORR), durable clinical benefit (DCB), OS, and progress-free survival (PFS) in individuals with high TMB as compared to those with low TMB. Single 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 CRC, 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 three 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. Specifically noted is 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 ≥ ten 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% CI, 21 to 39) and 57% of those responses lasting ≥ 12 months in individuals with TMB-high solid tumors (n = 102). Nine different tumor types were included. KEYNOTE-158 pre-specified ≥ ten and ≥ thirteen 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 F1CDx to include a CDx indication for TMB-high solid tumors using cut-point of ten 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 discussed in the 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 F1CDx with prespecified definition of TMB-high of at least 10 mut/Mb and participants received pembrolizumab 200 mg intravenously every three weeks for a maximum of 35 cycles. The primary outcome was the proportion of participants with a complete or partial response per RECIST v1.1. Objective responses were recorded in 29% (95% CI, 21 to 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 after 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. Key outcomes included ORR per RECIST as determined by blinded, independent radiology reviewers and investigators and locally assessed prostate specific antigen (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 (F1CDx)

F1CDx is an FDA-approved panel that is used as a CDx test to help identify individuals who might benefit from treatment (in accordance with FDA product labeling) with over 20 unique drug therapies. NGS-based CGP 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 TMB and MSI, and in ovarian cancer, genomic loss of heterozygosity (gLOH) are reported with F1CDx. Included in a 2022 clinical and analytical validation were multiple comprehensive evaluations of F1CDx including limit of detection, limit of blank, precision, and orthogonal concordance for short variants, copy number alterations, genomic rearrangements and select biomarkers. This assay validation including over 30,000 test results added to the growing body of evidence supporting clinical utility of F1CDx for matching individuals with solid tumors to targeted treatments based on their tumor's genomic variations and biomarkers. (Milbury et al., 2022)

In a prospective cohort study evaluating the role of CGP with F1CDx, Takeda et al. (2021) performed genomic testing on 181 tumor tissue samples from individuals with cytologically or histologically confirmed advanced or recurrent solid tumor cancers. Of the total samples, data was successfully obtained for 175 samples. Known and likely pathogenic actionable variations were found in 174 individuals (99%) and 24 of those (14%) received matched targeted therapy. *TP53* (n = 113), *PIK3CA* (n = 33), *APC* (n = 32), and *KRAS* mutations (n = 29) were the most common known/likely pathogenic variants found. Of 153 individuals evaluated for TMB, median TMB was four mutations/Mb. Tumors with high TMB (defined as ≥ 0 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 2024].

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 archived tumor tissue or new biopsies and then reviewed by a molecular tumor board to determine if any molecular-based therapies were available. At four different institutions, 2,579 participants were enrolled, and the tumor board reviewed 1,980 individual molecular profiles. Genes determined to be most frequently altered 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 subjects (27%); however only 163/2579 (6%) received at least one molecular-based recommended therapy. Out of the 182 lines of molecular-based recommended therapy started, 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. Further study of the use of broad genomic panels is currently in process in ongoing clinical trials.

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 2023 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.), 2023].

Guardant360 TissueNext™ (Guardant Health, Inc.)

Guardant360 TissueNext is a non-FDA-approved CGP 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 assess the evidence for the use of Guardant360 TissueNext [Hayes, Guardant360 TissueNext (Guardant Health Inc.), 2023].

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

In 2022 (updated 2024), Hayes published a Molecular Test Assessment on the MI Profile (Caris Life Sciences) for 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 whole exome sequencing), 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 in order 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 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, updated 2024].

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 whole exome sequencing. 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 (MMR) status found with the NGS platform with PCR fragment analysis for the same group. When compared with MSI 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), PPV 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

A liquid biopsy is a minimally invasive technique which uses bodily fluids (most commonly blood, but also urine, cerebrospinal fluid, pleural fluid, and other bodily aspirates) to analyze different types of biomolecules including ctDNA/cfDNA, RNA, circulating tumor cells (CTCs), proteins, methylation, and extracellular vesicles. Liquid biopsy results

can often be obtained quickly and can assist with therapeutic assessment, prognostication, and clinical decision-making without traditional biopsy (Nikanjam et al., 2022).

Bhave et al. (2024) investigated the clinical utility of CGP using tumor tissue and liquid biopsy in HR+, HER2- metastatic breast cancer in a retrospective study using records from a deidentified database containing clinical and genomic testing results of affected individuals who had received tissue or liquid biopsy at Foundation Medicine. The prevalence of several genomic alterations [*ESR1*mut, *PIK3CA*mut, *AKT1*mut, *PTEN*mut, and *PTEN* homozygous copy loss (*PTEN*loss)] was calculated for both tissue and liquid biopsies. Approximately 60% of HR+, HER2- metastatic breast cancer cases demonstrated one or more genetic alterations identified by tissue or liquid biopsy with ctDNA tumor fraction of at least 1% in the first line setting, whereas in liquid biopsy with ctDNA tumor fraction less than 1%, the prevalence dropped to 26.5%. *ESR1*mut frequency increased with each treatment line, especially when liquid biopsy was used; it demonstrated 59% prevalence when ctDNA tumor fraction was at least 1% in third line treatment. *PTEN*loss was found at significantly higher rates in tissue biopsy than liquid biopsy, which is consistent with the previously identified limitations of ctDNA testing. According to the authors, the study results support the procurement of a tissue biopsy for CGP at the time of recurrent/de novo diagnosis, followed by liquid biopsy to potentially identify acquired genetic alterations in second or greater lines of treatment. Also advised is the performance of reflex tissue biopsy if ctDNA tumor fraction is lower than 1%. Funding for this study was provided by manufacturers of CGP tests, and employees of the manufacturers were involved in the design and interpretation of the study and its results, which creates potential for bias. In addition, the study was limited by its retrospective design and included no participants with serial CGP.

In a retrospective study aimed to assess the feasibility and potential benefit of liquid biopsy performed simultaneously with tissue testing in a group of individuals newly diagnosed with stage IV lung adenocarcinoma, Maity et al. (2023) compared participants from a community-based academic medical institution who received tissue genotyping alone (standard biopsy) with participants who received simultaneous tissue and liquid genotyping (combined biopsy). A total of 120 participants met inclusion criteria and were evaluated in the study (78 in the standard biopsy group and 42 in the combined biopsy group). Participants were diverse in age, sex, race, and smoking status. For the standard group, the mean time to diagnosis was 33.5 days, while the combined group had a mean time to diagnosis of 20.6 days ($p < .001$ by two-tailed t-test). Fourteen of the participants in the combined group did not have sufficient tissue available for molecular testing, but for 79% of those (11/14), the liquid biopsy detected a genomic alteration, eliminating the need for another tissue biopsy. Importantly, in participants who completed both tests, each test found actionable alterations that had been missed by the other test; of the 28 participants in the combined group that successfully completed both tissue and liquid testing, 39% (n = 11/28) had informative genetic alterations detected with only one of the two tests performed. This is consistent with results of other studies performed at large academic centers. The authors concluded that simultaneous performance of liquid and standard tissue biopsy for genotyping could result in positive outcomes including shorter time to definitive molecular diagnosis, reduction in repeat tissue biopsies, and better detection of actionable genomic alterations. They also suggest that a sequential strategy that begins with liquid biopsy may be more cost-effective. Although the study results are promising, this study was small, retrospective, and observational, with no randomization, limiting the quality of the results. Additional study is recommended to determine the best genotyping strategy for individuals with stage IV lung adenocarcinoma.

Consensus recommendations published by Ionescu et al. (2022) addressed the use of molecular testing for targetable alterations in NSCLC. The recommendations were developed by a Canadian expert multidisciplinary team including participants representing medical oncology, pathology, and medical genetics who performed a targeted literature review to inform their recommendations. The group recommended testing at the time of initial diagnosis of non-squamous NSCLC for all targetable alterations (including new, as well as previously recognized standard-of-care, targetable alterations) as part of a comprehensive panel comprised of biomarkers that are incorporated in current Canadian consensus recommendations as well as international guidelines such as the National Comprehensive Cancer Network (NCCN) College of American Pathologists (CAP), the International Association for the Study of Lung Cancer (IASLC), and the Association for Molecular Pathology (AMP). These tests should have the ability to detect all mutation types that are applicable for targetable alterations, to include gene fusions, copy number variants, single nucleotide variants, and small insertions or deletions. In addition, comprehensive biomarker testing including new targets should be considered beyond adenocarcinoma for individuals that could have enhanced incidence of driver mutations, and when such detection could impact treatment decisions. For individuals with advanced NSCLC who develop resistance to targeted therapy, comprehensive biomarker testing should be performed/repeated. The panel indicates that liquid biopsy can be considered as either an alternative or complementary approach to tissue testing in individuals with advanced NSCLC and is preferred over tissue biopsy as a first step when an individual has had progression of disease after a targeted therapy. In this instance, liquid biopsy testing may help identify mechanisms of acquired resistance to targeted treatment. If a targeted alteration is identified via liquid biopsy, this may be considered actionable, however a negative result should be confirmed via tumor tissue biopsy. The panel goes on to recommend that molecular tumor boards be established to assist providers with the interpretations of results and selection of the most appropriate treatments.

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 NSCLC were identified and values were extracted identifying the number of true-positive, false-positive, false-negative, and true-negative results. 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). In addition, the meta-analysis showed high specificity and area under curve (AUC) > 0.9, demonstrating a general high diagnostic efficacy in the exposure of KRAS mutations by cfDNA investigation. The values of the likelihood ratios [PLR 8.32 (95% CI, 6.93–9.99) and NLR 0.29 (95% CI 0.26–0.33)] 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. Publications by Thompson et al. (2016), Sacher et al. (2016), and Leighl et al. (2019), previously discussed in this policy, were included in the Palmieri systematic review.

Hayes Precision Medicine Insights reports addressed 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 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. Whole exome sequencing 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, whole exome sequencing 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 CRC 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 CRC. After review, 69 studies were included and 17 studies reviewed total cfDNA, six studies looked at the DNA integrity index and 15 focused on ctDNA. While the researchers concluded that ctDNA is a promising candidate for CRC screening, further study is required.

A study on renal cell carcinoma (RCC) by Yamamoto et al. (2019) evaluated ctDNA for clinical utility. Fifty-three individuals with histologically diagnosed with clear cell RCC were enrolled and sequencing was performed on plasma cfDNA and tumor DNA. A total of 38 mutations across 16 (30%) participants were identified from cfDNA, including mutations in TP53 (n = 6) and VHL (n = 5), and median mutant allele frequency of ctDNA was ten percent. 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 individuals with LUSC; 410 participants (stage 3B or 4 LUSC) were tested with a targeted cell-free circulating DNA NGS assay and 82 participants (any stage) were tested with a tissue NGS cancer panel. Overall, 467 subjects (95%) had a diagnosis of LUSC, and 25 (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 participants 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 *ALK*, *BRAF*, *EGFR*, *ERBB2*, *KRAS*, *MET*, *ROS1*, *STK11* and 26 other genes in individuals with NSCLC. 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 CNVs were found. Three splice site variants were found. Digital PCR (dPCR) was performed on these three and a *TP53* mutation was confirmed in one, but not the other two. A further 92 samples from healthy donors and 242 samples from untreated individuals with NSCLC were tested, and these three variants were not seen. In the affected group, twenty participants with NSCLC were tested by both InVisionFirst and dPCR at two separate labs, blinded to each other's results. In this cohort, 40% of subjects 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 used to simulate a wide array of copy number and other genetic variations were tested in the same way. Overall, in the donor samples and contrived materials, the concordance rate between InVisionFirst and dPCR was high. InVisionFirst demonstrated a > 99% sensitivity for SNVs and > 92% for indels.

Sun et al. (2018) published a study examining liquid biopsies in CRC. The researchers analyzed blood from 140 individuals with CRC using matched tumor samples. Both CTC and 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 individuals 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 CTC first. The study concluded that using CTC and ctDNA for monitoring molecular profile changes in individuals with CRC may be useful.

A study from Dieffenbacher et al. (2018) evaluated tumor tissue and liquid biopsies in individuals with metastatic clear cell RCC 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 individuals with RCC.

Cohen et al. (2017) conducted a cohort study to develop a noninvasive test for detection of pancreatic ductal adenocarcinoma. The researchers combined blood tests for KRAS gene mutations with protein biomarkers as a testing method. They tested this assay on a cohort of 221 study participants with resectable pancreatic ductal adenocarcinomas and 182 control participants without known cancer. In the plasma samples of 66 subjects (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 participants with gastric cancer (78%), and 25 participants (33%) had targetable alterations (ERBB2, n = 11; MET, n = 5; FGFR2, n = 3; PIK3CA, n = 6). In NSCLC, 62 participants (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 participants that had tissue available for confirmation (ten 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.

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.

A 2023 Hayes Molecular Test Assessment assessed the clinical validity and utility of the FoundationOne Liquid CDx test when used as a CDx for specific cancer treatments or for tumor mutational profiling for individuals with solid tumor cancer. A total of seven studies met inclusion criteria and were evaluated for the report; all studies were determined to have very poor-quality. Evidence provided by these studies suggest that FoundationOne Liquid CDx may help with determination of the eligibility of impacted individuals for various treatments, which can be especially helpful for individuals with advanced cancer diagnoses; however the studies did not determine conclusively whether comprehensive testing can lead to better clinical outcomes when compared with more limited testing, nor did they provide clarity regarding the use of cfDNA testing used preferentially over tissue biopsy test. The Hayes report indicates that weak support is provided by existing guidelines and position statements with regard to the use of ctDNA testing but notes that the test is FDA-approved as a CDx for breast cancer, CRC, prostate cancer, NSCLC, ovarian cancer and solid tumors in individuals who may benefit from treatment with targeted therapies in accordance with the approved product labeling. Of note, only 12 of the 324 genes included in FoundationOne Liquid CDx are incorporated in the FDA-approved CDx indications and it is not approved for assessment of TMB or MSI, though the manufacturer indications the test is validated for detection of these biomarkers.

Bayle et al. (2023, included in the 2023 Hayes FoundationOne Liquid CDx Molecular Test Assessment) 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 PFS was 4.7 months (95% CI, 2.7-6.7 months), and median OS was 8.3 months (95% CI 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.

The FoundationOne CDx panel (either tissue or liquid biopsy) was performed on specimens from 203 individuals with cancer in a prospective, single-center study by Pinet et al. (2023, included in the 2023 Hayes FoundationOne Liquid CDx Molecular Test Assessment). The primary goal was to improve understanding of the real-world impact of standard use of FoundationOne for individuals with cancer and a poor prognosis/limited treatment options or individuals whose treatment progressed after a minimum of one course of standard treatment. Secondary aims were: evaluation of the feasibility of using FoundationOne tests in terms of rate of failure, assessment of the rate of detection of targetable and non-targetable abnormalities, assessment of the rate of targeted treatment based on results of testing, and lastly, evaluation of the OS of individuals receiving targeted therapy compared to those who did not. After application of exclusion criteria, 162 participants were included in the cohort. Successful results were obtained for 93% (n = 150) of the participants. A total of 2,419 gene variations were detected; median number of variations per tumor was 11 (range 0-86). Common or likely pathogenic variants were detected most frequently in TP53, TERT, PI3KCA, CDKN2A/B, KRAS, CCDN1, FGF19, FGF3, and SMAD4. Of participants with TMB available, the median TMB was three/Mb (range 0-117). Thirteen participants (8.6%) received matched targeted therapy based on known or likely pathogenic variations. Of 69 participants whose cases went to a Molecular Tumor board for evaluation, 60 participants received treatment recommendations. OS was not significantly impacted by genotype-directed treatments [13 months with genotype-directed treatment vs. 14 months without genotype-directed treatment; p = 0.95; hazard ratio = 1.04 (95% CI, 0.48-2.26)]. Based on these outcomes, the

researchers indicate that a well-organized facility including a Multidisciplinary Molecular Tumor Board and availability of NGS screening can produce results similar to those of larger cancer centers related to appropriate enrollment in clinical trials. The primary limitations to pursuing genetically-guided therapies were the clinical condition of the affected individual and available access to drugs. Limitations included small size, lack of randomization and a mixed population of cancer types. Larger, randomized clinical trials with focus on specific cancer types are recommended.

To investigate the relationship between ctDNA tumor fraction and the identification of actionable genomic alterations across various cancer types, Husain et al. (2022, included in the Hayes 2023 FoundationOne Liquid CDx Molecular Test Assessment) evaluated a consecutive series of liquid biopsies [performed with FoundationOne Liquid CDx (F1LCDx)] conducted in the United States during routine clinical care. Specimens from a total of 23,482 individuals with 25 solid tumor types were evaluated. The primary outcome assessed was the prevalence of targeted alterations according to cancer type and detection as related to ctDNA. Also evaluated was the sensitivity of liquid biopsy detection in a group of 1,289 participants with tissue testing results available. Overall, detectable ctDNA was found in 94% (n = 22,130) of liquid biopsy samples and the median tumor fraction was 2.2%. Genetic alterations in NCCN category one genes were detected by liquid biopsy in 37% of individuals with lung cancer, 30% of individuals with prostate cancer, 36% of individuals with breast cancer and 51% of individuals with colon cancer. In samples with tumor fraction of at least ten percent, sensitivity of liquid biopsy to detect driver alterations that had been found in tumor tissue analysis from the same individual were consistently near or at 100%. Samples with lower tumor fraction ranged in sensitivity from 58% to 86%. Based on these results, the authors assert that CGP of ctDNA is a practical approach to the detection of guideline-associated actionable genomic alterations for various cancer types, and elevated ctDNA shedding relates to both high sensitivity and high negative predictive value for the identification of actionable genetic alterations. When liquid biopsy specimens have higher tumor fraction, it is likely the results are sufficient and may lead to a reduction in reflex to confirmatory tissue testing when negative liquid biopsy results are obtained. Although the evidence base supporting improvement in clinical outcomes related to use of ctDNA detected biomarkers is growing, this study is limited by its retrospective design and does not directly evaluate whether liquid biopsy profiling improves clinical outcomes when incorporated into routine clinical care.

Caputo et al. (2022) used FoundationOne Liquid Analysis [either FoundationOne Liquid (70 genes) or FoundationOne Liquid 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. 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 ORR; secondary outcomes included independent review facility-assessed ORR, duration of response, PFS, 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% 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 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 more than 7,500 tests and at least 30,000 individual variants over more than 300 genes and 30+ types of cancer. The results of this analysis showed 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 8,000. Reproducibility of variant identification was 99.59%. Overall positive percent agreement and negative percent agreement of 96.3% and over 99.9%, respectively, was observed. The authors concluded that FoundationOne Liquid CDx is accurate with reproducible results and can reliably detect the main types of genomic alterations as well as complex biomarkers (e.g., MSI, blood TMB, and tumor fraction).

Guardant360® and Guardant360® CDx

Guardant360 and Guardant360 CDx (Guardant Health, Redwood City, CA) are ctDNA-based CMP tests designed to aid in treatment selection for individuals with advanced solid tumors. Guardant360 CDx is a 55-gene panel that is FDA-approved as a CDx for use in the treatment of non-small cell lung cancer, breast cancer, and for general tumor mutation profiling for any solid tumor cancer. Guardant360 is an expanded version of Guardant360 CDx. Guardant360 is not FDA-approved and is marketed for use in guiding treatment selection in individuals with advanced stage cancer who have progressed on therapy. Guardant360 assesses markers that are related to ICI therapy and includes analysis of variants in 83 genes, additional single nucleotide alterations, insertions, and deletions, copy number amplifications, and fusions as well as TMB, MSI, and homologous recombinational repair.

A retrospective, observational study led by Powell et al. (2024) sought to evaluate the relationship between optimal first-line treatment for 359 individuals with advanced NSCLC and ALK rearrangement/EGFR mutations and clinical outcomes. The GuardantINFORM database was used to obtain genomic information as well as claims for individuals with advanced or metastatic NSCLC. Participants included in the evaluation were categorized as either having optimal or suboptimal first-line therapy, as determined by use of CGP findings (in this study, Guardant360 was used). Claim information was used to ascertain real-world time to next treatment, real-world time to discontinuation of treatment, and use of health services including emergency room, inpatient treatment, and outpatient treatment in the 12 months after starting first-line therapy. The researchers determined that 78% of participants (280/359) received optimal first-line therapy. These individuals had longer median real-world time to next treatment (11.2 vs 4.4 months; $p < .01$) as well as real-world time to discontinuation (10.4 vs 1.9 months; $p < .01$). The optimal group also had substantially fewer emergency department and outpatient visits (0.76 vs 1.27; $p < .01$ and 22.9 vs 42.7; $p < .01$, respectively) than the suboptimal group, although no significant difference was found in inpatient care occurrences. From this data, the authors determined that individuals with NSCLC who received optimal treatment per CGP using NGS-based ctDNA testing had better clinical and utilization outcomes, which supports the use of genomic profiling prior to treatment of advanced or metastatic NSCLC as recommended by existing guidelines including NCCN and ASCO.

In a 2024 Molecular Test Assessment, Hayes explored the evidence on Guardant360 and Guardant360 CDx test and evaluated the utility of these tests for use in directing treatment clinical decision-making in individuals with advanced solid tumor cancers. Overall, Hayes identified a low-quality body of evidence for use of Guardant360 and Guardant360CDx for the identification of targeted therapy in individuals with advanced non-small cell lung cancer and a very low-quality body of evidence for individuals with metastatic breast cancer and other types of advanced solid tumors. The report notes, however, that there are factors which complicate evidence-based decision making related to the use of Guardant360 and Guardant360 CDx; though there may be a lack of direct evidence demonstrating survival benefit associated with these tests, there is implied clinical utility in certain circumstances, such as seeking specific genetic variants in solid tumors to facilitate use of FDA-approved targeted treatments, testing for TMB or MSI in individuals who may be eligible for ICI therapy, and the use of these liquid-based tests in individuals with insufficient tumor biopsy samples or for whom a biopsy is not feasible. [Hayes, Guardant360 and Guardant360 CDx (Guardant Health, Inc.), 2024].

To investigate whether immediate testing of plasma ctDNA along with standard tissue testing could potentially shorten the time to implementation of treatment and improve clinical outcomes compared to tissue testing alone in individuals affected with suspected advanced NSCLC, Yang et al. (2023, included in the Hayes Guardant360 and Guardant360 CDx Molecular Test Assessment) conducted a prospective, randomized trial. A total of 180 participants were enrolled and randomized into two groups. After clinical tumor workup, individuals who were found to have benign disease, small cell lung cancer, early-stage NSCLC, or any other type of cancer were excluded. Group A, comprised of 63 participants and Group B, comprised of 59 participants were included in the trial analysis. All were suspected to have advanced NSCLC and underwent liquid biopsy with NGS testing (using Guardant360) at their first visit, then went on to undergo standard histological diagnosis along with tissue genotyping. Group A received their NGS results after tissue genotyping and Group B received NGS results as soon as possible after histological diagnosis of advanced NSCLC was confirmed. Time to start of systematic treatment was the primary outcome and secondary outcomes were biomarker discovery, objective response rate, and PFS. Most participants had adenocarcinoma (77.8% in Group A and 79.7% in Group B). The rate of *EGFR* variations was similar in Group A and Group B (57.1% vs 56.6%, respectively), and other driver mutations were uncommon. The researchers determined that median time to treatment was shorter for Group B (20 days) than Group A (28 days). The objective response rate and PFS rates did not differ significantly between the two groups. Concordance between liquid NGS and tissue NGS was high. Notably, liquid NGS identified driver mutations in 20/47 (42.6%) cases

where tissue testing was negative. Although this study was small and lacked the power to detect differences in survival outcomes, the researchers suggest that their results support the use of liquid NGS at the initial clinic visit for suspected advanced NSCLC to provide complete genotyping quickly to identify individuals who may benefit from targeted treatment and shorten the time to treatment initiation.

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 and 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 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% and emerging and potentially targetable mutations were found in 40.1%. In participants whose ctDNA testing showed qualifying alterations, time to discontinuation of therapy and OS 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 Guardant360 CDx as a blood-based CDx 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 duration of response (DOR), disease control rate (DCR), and time to response (TTR) in individuals with *KRAS* p.G12C–mutant NSCLC as detected by Guardant360 CDx comparative to the whole analysis group. The ORR (95% CI; individuals with objective response/all individuals in the dataset) for all individuals was 37.1% (28.6%, 46.2%; n = 46/124) in the Full Analysis Set, 36.4% (25.7%, 48.1%; n = 28/77) in the Guardant360 positive cohort, and 46.7% (28.3%, 65.7%; n = 14/30) in the Guardant360 negative cohort. Rates of progressive disease (PD), stable disease (SD), and partial response (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%) of those in the full analysis set and 24/28 (85.7%) in the Guardant360 positive cohort; DOR was ≥ 6 months in 28/46 (60.9%) and 15/28 (53.6%) of those in the full analysis set and Guardant360 positive cohort, respectively. Of the four cohorts, DOR ≥ three months among responders was numerically highest in the Guardant360 positive cohort [n = 24/28 (85.7%)], while DOR ≥ 6 months was mathematically highest in the Guardant360 negative [n = 9/14 (64.3%)] cohort. The average time to objective response was comparable between all cohorts. The authors concluded that liquid biopsy using Guardant360 CDx has clinical validity for the identification of individuals with KRAS p.G12C-mutant NSCLC and, amplified by tissue testing methodologies, will identify individuals for treatment with sotorasib.

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 individuals with *ROS1* 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 fusions for seven individuals 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 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 participants 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 plasma 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 individuals with advanced NSCLC and tumors that carried *ALK* gene fusions. The researchers sought to analyze cfDNA to find a non-invasive way to identify these gene fusions. The study used the Guardant360 database of NSCLC cases to identity participants. Eighty-eight individuals with 96 plasma-detected *ALK* fusions were identified. The fusion partners detected 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 as well as other solid tumors. Plasma from individuals 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 detected. Additionally, mutations in *BRCA1*, *BRCA2*, and *ATM* were found in 18.1% (32/177) of cases. The researchers compared ctDNA and tumor tissue results 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 individuals diagnosed with NSCLC undergoing analysis of ctDNA using Guardant360. As part of clinical care, 90 individuals were submitted for ctDNA testing, but only 68 provided the necessary consent. These participants had lung adenocarcinoma (n = 55, 81%), lung squamous cell carcinoma (n = 12, 17.7%) and other lung cancers (n = 1, 1.3%). Of the 68 subjects, 38 were tested using a 54-gene ctDNA panel and 31 underwent testing with a 68-gene ctDNA panel. Tissue-based testing was performed on 44 subjects using nine 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 participants had matched tissue and blood samples, and, in those subjects, an EGFR activating mutation was found in both tissue and blood in five paired samples, and in tissue only in two samples (71% concordance). In nine subjects with paired tissue and blood samples, an EGFR driver mutation was identified in both plasma and tissue in five participants, plasma only in one participant, and in tissue only in three participants. 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)

A 2024 ASCO Rapid Recommendation Update (Burstein et al., 2024) addressed emerging evidence regarding endocrine and targeted treatments for HR+, HER- metastatic breast cancer. This update focused on the CAPItello-291 phase III, double-blind, randomized controlled trial, which analyzed fulvestrant with the AKT pathway inhibitor capivasertib3 and led to the US Food and Drug Administration approval of capivasertib and a CDx test on November 16, 2023. A similar study, FAKTION, which was a randomized phase II comparison of fulvestrant with either capivasertib or a placebo, resulted in outcomes similar to CAPItello-291, showing benefit in tumors with only *PIK3CA/AKT1/PTEN* variations. In this update, the expert panel recommended multiple lines of endocrine treatment, often paired with targeted agents identified by prior treatments as well as routine testing for activating mutations in *PIK3CA, AKT1*, or *PTEN* (evidence quality: high, strength of recommendation: strong).

In 2023, Burstein et al. published an ASCO guideline Rapid Recommendation Update indicating that routine testing for emergence of *ESR1* mutations at recurrence or progression on endocrine therapy (with or without CKD4/6 inhibitor) is advised for individuals with ER+, HER2- metastatic breast cancer. Testing should be performed on blood or tissue obtained at the time of progression. The use of blood-based testing is preferred due to greater sensitivity and if tumor or ctDNA test shows *ESR1* wild-type, additional testing at subsequent progression may be necessary (evidence quality: high, strength of recommendation: strong).

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 NSCLC (strength of recommendation: strong)
- In individuals with advanced or metastatic solid tumors, genomic testing should be considered in order to determine whether the individual 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. ASCO goes on to suggest that repeat genomic testing may be undertaken for individuals who have developed resistance to targeted therapies, especially when known acquired resistance mechanisms might have an impact on next-line treatment choice, or for the purpose of identifying new targets in tumors when an individual has had progression or after prolonged, stable disease on targeted therapies.

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 such 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.

An update to the ASCO guideline addressing endocrine treatment and targeted therapy for HR+, HER2- metastatic breast cancer (Burstein et al., 2021) recommends using NGS in tumor tissue or cfDNA in plasma for the detection of PIK3CA mutations to guide decisions around the use of alpelisib combined with fulvestrant in postmenopausal individuals and in males with HR+ metastatic breast cancer (evidence quality: high, strength of recommendation: strong).

Merker et al. (2018) published a joint review from the ASCO and the College of American Pathologists (CAP) assessing the clinical use of 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.

European Society for Medical Oncology (ESMO)

ESMO's Precision Medicine Working Group updated their recommendations for the standard use of tumor NGS for individuals with advanced cancers in 2024 (Mosele et al.) The ESMO Scale for Clinical Actionability of molecular Targets (ESCAT) was used in the development of ESMO's updated recommendations, with focus placed on genomic alterations classified as ESCAT level I (ready for routine use, based on clinical evidence). ESCAT level II alterations were also reported in this document in order to facilitate enrollment of affected individuals in clinical trials and to promote the development of targeted drugs. Overall, ESMO recommends performing tumor NGS in individuals with advanced cancers including non-squamous NSCLC, breast cancer, CRC, prostate cancer, and ovarian cancer. Additionally, for rare tumors, tumor NGS is recommended in individuals with advanced cholangiocarcinoma, gastrointestinal stromal tumors, sarcoma, thyroid cancer, and unfavorable cancer of unknown primary. For the detection of tumor-agnostic variations in individuals with advanced cancers, ESMO recommends tumor NGS when tumor-agnostic targeted treatment is available in the country in which the affected individual is being treated/resides. The importance of ensuring applicable fusions are included in the test panel administered is highlighted.

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.

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 NSCLC and advanced stage 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 PFS vs. clinical decisions alone. Additionally, some evidence demonstrated that CMI may lead to improved OS. 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 biomarker testing for specific cancer types including the use of multigene panels and molecular profiling. NCCN specifically mentions liquid biopsy (plasma) testing in certain clinical scenarios as well.

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 including, but not limited to, fusions (ALK, NRG1, NTRK, ROS1, FGFR2, RET), mutations (BRAF, BRCA1/2, KRAS, PALB2), amplifications (HER2), MSI, dMMR or TMB via an FDA-approved and/or validated NGS-based assay is recommended. For identifying RNA fusions, RNA sequencing assays are preferred because gene fusions are better identified by RNA-based NGS. Testing on tumor tissue is preferable, but cfDNA testing can be considered if tumor tissue testing is not feasible. (NCCN Ampullary Adenocarcinoma, v2.2024)

Biliary Tract Cancers

Biliary tract cancers are associated with clinically relevant molecular alterations that are expressed in different manners in gallbladder cancers and intrahepatic and extrahepatic cholangiocarcinomas. Specifically, translocations in *FGFR2* and *NTRK*, mutations in the *IDH1* and *BRAF* genes and MSI, along with other rare molecular alterations, have been detected by genotyping of the tumor tissue. Targeted treatment for these situations are now available. Therefore CMP is recommended for all individuals with unresectable or metastatic biliary tract cancers who are candidates for systematic therapy. Repeat biopsy may be necessary depending on accessibility of the tumor, safety, and clinical context. cfDNA testing may be considered, but may not reliably identify gene fusions or rearrangements, dependent on the specific panel and partner gene. (NCCN Biliary Tract Cancers, v4.2024)

Bladder Cancer

For bladder cancer, NCCN recommends molecular/genomic testing (in a CLIA-approved laboratory), including FGFR RGQ RT-PCR for *FGFR3* genetic alterations and IHC for HER2 overexpression, 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, v4.2024)

Bone Cancer

NCCN Bone Cancer guidelines recommends consideration of CGP via validated/FDA-approved assay for individuals with metastatic chondrosarcoma, recurrent chordoma, metastatic Ewing sarcoma and metastatic osteosarcoma to identify potential targeted treatment opportunities and encourages impacted individuals to participate in well-designed clinical trials to further advance study. Ninety percent of individuals with Ewing sarcoma will have one of four specific cytogenetic translocations; if negative for these, additional molecular testing is recommended (NCCN Bone Cancer, v1.2025).

Breast Cancer

The NCCN guideline for Breast Cancer indicates that genomic profiling may be performed for use in determining appropriate treatment for breast cancer. Either tumor tissue or plasma-based ctDNA assays can be used; each of these provide both benefits and limitations in terms of diagnosis and progression of disease. Tissue based assays are higher in sensitivity, but ctDNA may more accurately reflect tumor heterogeneity. If one sample is negative for actionable biomarkers, providers may consider testing on the alterative specimen type. In the setting of recurrent unresectable or stage IV breast cancer, testing for biomarkers associated targeted 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), and if liquid biopsy is negative, tumor tissue testing is recommended. (NCCN Breast Cancer, v4.2024)

Central Nervous System Cancers

The NCCN guideline for central nervous system (CNS) cancers (v2.2024) considers the incorporation of relevant diagnostic markers, including both histopathologic and molecular information, standard practice for classification of tumors. NGS is the preferred approach for pathologic evaluation of CNS tumors due it its ability to screen for multiple diagnostic and prognostic alterations in one test.

Cervical Cancer

For persistent or recurrent cervical cancer, NCCN indicates

- CGP as determined by an FDA approved assay or a validated test performed in a CLIA-certified laboratory 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, v3.2024)

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. Universal MMR or MSI testing is recommended for all individuals newly diagnosed with colon or rectal cancer. For individuals with metastatic CRC, recommended workup should include determination of tumor gene status for *KRAS/NRAS* and *BRAF* mutations, either individually or as part of an NGS panel test (preferred). For suspected or proven metastatic adenocarcinoma or rectal cancer, *RAS* and *BRAF* mutations should be assessed as well as HER2 amplifications and MMR or MSI status if not previously evaluated. This testing should be conducted as part of broad molecular profiling to detect rare and actionable mutations and fusions. NGS panels have the advantage of the ability to detect rare and actionable gene alterations such as *NTRK* and *RET* 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 detect rare and actionable mutations and fusions. Repeat molecular testing should not be performed after standard cytotoxic chemotherapy as important molecular changes rarely occur in this situation. Molecular profile changes are more common after targeted therapy; repeat testing can be considered to guide future decisions regarding targeted therapies. (NCCN Colon Cancer, v5.2024, NCCN Rectal Cancer, v4.2024)

Gastric and Esophageal/Esophagogastric Junction Cancers

Several targeted agents have been approved by the FDA for use in gastric, esophageal, and esophagogastric junction cancers. Immunohistochemistry, in situ hybridization, and/or targeted PCR testing should be employed as first line tests for identification of biomarkers, followed by NGS testing. When limited tissue is available, 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 will exhaust the material available for testing. In these situations or per the discretion of the treating provider, CGP with a validated NGS assay (performed in a CLIA-approved laboratory) may be used to identify applicable biomarkers (such as HER2 overexpression/amplification, MSI status, TMB, *NTRK*, and *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 or esophageal/esophagogastric cancers 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. Regarding repeat biomarker testing, NCCN advises that repeat testing may be considered with clinical or radiologic progression for individuals with advanced/metastatic gastric or esophageal/ esophagogastric junction cancer. (NCCN Gastric Cancer, v4.2024, NCCN Esophageal and Esophagogastric Junction Cancers, v4.2024)

Gastrointestinal Stromal Tumors

NCCN recommends molecular testing for potential driver mutations in gastrointestinal stromal tumors. Tissue biopsy is preferred for this testing, but ctDNA may be appropriate for specific cases. Molecularly guided treatment is the primary therapy for metastatic gastrointestinal stromal tumors. (NCCN Gastrointestinal Stromal Tumors, v2.2024)

Histiocytic Neoplasms

NCCN recommends molecular mutation profiling to aid with confirmation of a clonal Langerhans or histiocytic process and to detect potentially important mutations or therapeutic targets. For Langerhans cell histiocytosis and Erdheim-Chester disease, molecular tests for somatic mutations or fusions can be performed in a stepwise fashion or in parallel. Somatic NGS panel testing should detect the common MAPK pathway mutations and RNA-based molecular panel fusion tests can detect *BRAF, ALK, RET*, and *NTRK1* rearrangements. If molecular testing is negative, repeat testing, potentially with a new sample, is recommended. In individuals suspected of having Rosai-Dorfman disease or histiocytosis and a 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, v2.2024)

Hepatocellular Carcinoma

A range of molecular alterations are associated with hepatocellular carcinomas. At this time, however, no treatments with differential benefits for specific molecularly identified subgroups of hepatocellular cancers have been identified. Although there is not an established indication for routine genetic profiling, molecular testing can be considered on a case-by-case basis, and clinical trial participation is encouraged. Tumor profiling should be considered for all individuals with advanced

stages of mixed hepatocellular cancer and cholangiocarcinoma to detect potential targetable alterations that may be associated with cholangiocarcinoma. (NCCN Hepatocellular Carcinoma, v2.2024)

Melanoma: Cutaneous

Per the NCCN Cutaneous Melanoma guideline, several somatic genetic variations have been identified in cutaneous melanoma. Some of these are targetable driver mutations that can inform treatment decisions and/or eligibility for clinical trials. NGS allows DNA and RNA sequencing to be performed more quickly and is less costly than Sanger sequencing, although single gene or small multi-gene panels can be used in some cases to test an individual gene (e.g., BRAF) or a limited number of genes. Tumor tissue is preferred for molecular testing, but liquid biopsy may be performed if tumor tissue is not available. A negative liquid biopsy, however, should prompt a tissue test. In cases where initial presentation is stage IV disease or clinical recurrence, broader genomic profiling is recommended, if possible, specifically if the results of testing may help guide future therapeutic decisions or eligibility for clinical trial participation. Repeat testing upon recurrence or metastasis in individuals with melanoma is likely to be low yield unless new or more comprehensive testing methodologies are used or if there is a better sample available. Repeat testing following progression on targeted therapy does not appear to have clinical utility at this time, since the mechanisms of resistance are diverse and have no prognostic or therapeutic relevance. In some situations where tests with lower sensitivity or specificity were used, or if testing was only for specific mutations, repeat testing with a different method may be warranted. (NCCN Melanoma: Cutaneous, v2.2024)

Neuroblastoma

The NCCN guideline addressing neuroblastoma (NCCN, Neuroblastoma, v2.2024) states that currently, risk stratification for primary treatment is largely dependent on molecular tumor profiling, and molecularly-targeted therapies for pediatric solid tumors, including neuroblastoma, are emerging. NGS is recommended for concurrent analysis of *MYCN* amplification, segmental chromosomal aberrations (SCAs), and *ALK* alterations as long as the panel comprehensively assesses of copy number status and provides coverage of the pertinent regions of *ALK* and other neuroblastoma-associated genes.

Neuroendocrine and Adrenal Tumors

For individuals with neuroendocrine tumors and locoregional or unresectable/metastatic disease, tumor/somatic molecular profiling is recommended as a consideration for the detection of actionable variations that may assist with clinical-decision making for targeted treatments. Tumor tissue testing is preferred, but cfDNA testing is an option if tumor tissue testing is not feasible. (NCCN, Neuroendocrine and Adrenal Tumors, v2.2024)

Non-Small Cell Lung Cancer (NSCLC)

NCCN provides the following information regarding the principles of molecular and biomarker assessments in NSCLC (Non-Small Cell Lung Cancer, v9.2024):

- 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.
- Many available NGS-based assays are larger than the 50-gene limit in the CPT coding convention; as such, panels including more than 50 genes may be considered practical for following testing recommendations.
- Real-time PCR can be used in a highly targeted manner, assessing for specific mutations.
- Unmodified Sanger sequencing is not an appropriate choice for the identification of mutations in tumor samples with less than 25% to 30% tumor after enrichment and is not appropriate for those assays in which detection of subclonal events (resistance mutations) is important. With use of Sanger sequencing, tumor enrichment methods are almost always recommended.
- Methodologies that seek to evaluate sequences other than a subset of very specific variations (e.g., Sanger sequencing or NGS) can potentially identify VUS; these should not be considered as a basis for the selection of targeted therapy.
- FISH analysis may have better sensitivity for gene amplification in some situations.
- If an assay has a technical failure related to insufficient quantity, alternate testing modalities or the procurement of additional tissue is recommended.
- The use of peripheral blood (e.g., ctDNA) can be used in conjunction with tissue-based testing to achieve the appropriate genotyping for recommended biomarkers.

In the setting of progression on targeted therapy:

- When individuals receiving targeted therapy have disease progression, tissue biopsy should be considered for morphology and biomarker evaluation.
- Broad genomic profiling may be the most helpful approach to determining potential mechanisms of resistance; this could require more than one instance of genomic profiling during the course of therapy.

ctDNA testing:

- The use of ctDNA testing should not be used in lieu of a histologic tissue diagnosis.
- ctDNA testing is not typically recommended in situations other than advanced or metastatic disease. Tissue based testing is preferable in stage I-III cancer.
- Both ctDNA and tissue testing have very high specificity, but considerable false-negative rates, which supports complementary use of these methods. Complementary use can also reduce turnaround time and increase the identification of targetable alterations.
- 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.

Occult Primary Cancers

NCCN guidelines for occult primary cancers (NCCN, Occult Primary, v2.2025) advises that molecular profiling of tumor tissue using NGS (or other method which can identify gene fusions) may be considered for suspected metastatic malignancies after initial determination of histology to potentially detect uncommon mutations in individuals who are candidates for anti-cancer treatments. Tumor tissue testing is preferred, but cfDNA testing may be an option if testing of tumor tissue is not possible. Tissue of origin studies are not recommended. Currently, high-level evidence supporting the use of targeted therapies based on NGS for improved outcomes is limited.

Ovarian Cancer/Fallopian Tube Cancer/Primary Peritoneal Cancer

The current NCCN guidelines recommend tumor molecular analysis in a CLIA-approved facility in both the upfront setting and with recurrence of disease for ovarian cancer, fallopian tube cancer, or primary peritoneal cancer. In the initial setting, tumor molecular analysis should optimize detection of molecular variations that can impact decision-making regarding treatments that have demonstrated benefit, including *BRCA1/2*, loss of heterozygosity, or homologous recombination deficiency status in the absence of a germline *BRCA* mutation. For recurrent disease, NCCN recommends tumor molecular evaluation via validated test(s) 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, HER2 status, HRD status, MSI, MMR, TMB, BRAF, FRα (FOLR1), 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. This testing should be performed on the most recently tumor tissue available. When tissue-based testing is not clinical feasible, ctDNA may be used. (NCCN Ovarian Cancer/Fallopian Tube Cancer/Primary Peritoneal Cancer, v3.2024)

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. The use of RNA-sequencing assays are preferable for detection of RNA fusions because RNA-based NGS tests have greater ability to detect gene fusions. Of note, testing on tumor tissue is preferred; however, cfDNA testing can be considered if tumor tissue testing is not feasible. (NCCN Pancreatic Adenocarcinoma, v3.2024)

Prostate Cancer

Currently, tumor molecular and biomarker evaluation can be used to inform treatment in prostate cancer, including assessing eligibility for biomarker-directed therapies, genetic counseling, early use of platinum-based chemotherapy, and eligibility for clinical trial participation. Tumor molecular profiles may transform after treatment; thus re-evaluation may be considered at the time of cancer progression to assist with treatment decisions. Metastatic biopsy for histologic and molecular evaluation is strongly recommended by NCCN for prostate cancer. Biopsy can include lymph node for individuals with N1 disease. When biopsy is unsafe or not feasible, plasma ctDNA is an option for testing, 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. If MSI testing is used, an NGS assay that has been validated for prostate cancer is preferred. (NCCN Prostate Cancer, v4.2024)

Small Cell Lung Cancer

The NCCN Small Cell Lung Cancer guideline (NCCN, v 2.2025) indicates that CMP using blood, tissue or both may be considered in rare cases of small cell lung cancer – in particular, testing may be helpful for individuals with extensive stage/relapsed small cell lung cancer "who do not smoke tobacco, lightly smoke, have a remote history of smoking or have diagnostic or therapeutic dilemma, or at time of relapse—if not previously done, because this may change management".

Thyroid Carcinoma

For anaplastic thyroid carcinoma, molecular testing for actionable mutations should include *BRAF, NTRK, ALK, RET*, MSI, dMMR, and TMB. *BRAF* IHC testing is recommended due to faster turnaround compared to genetic testing. The NCCN Panel recommends molecular testing to help with clinical decision-making for systemic therapy and to determine eligibility for clinical trials. In advanced thyroid carcinoma, molecular testing has been shown to be helpful when choosing targeted treatment, particularly related to drug therapies or clinical trial participation. In addition, the presence of some mutations may have prognostic importance. (NCCN Thyroid Carcinoma, v4.2024)

Uterine Neoplasms

Per the NCCN Uterine Neoplasms Guidelines (v2.2024), molecular evaluation of endometrial carcinoma has detected four molecular subgroups with clinical significance (and varying clinical prognoses). Retrospective investigations have revealed that these four subgroups may respond differently to treatment. Prospective randomized trials are currently underway to determine the role of a molecularly-guided treatment strategy for high-intermediate risk and high-risk endometrial carcinomas. For metastatic uterine sarcoma, CGP provides helpful information for predicting rare pan-tumor targeted therapy opportunities and should include, at a minimum, *NTRK*, MSI, and TMB. Comprehensive molecular testing is strongly encouraged in the initial evaluation of uterine neoplasms. Dependent on results, clinical trial enrollment may be encouraged.

Vaginal Cancer

NGS and CMP profiling is recommended for consideration for individuals with recurrent or persistent disease to assist with selection of the most appropriate systemic therapy. Tissue biopsy is preferred for testing, but if such tissue is not available or biopsy is not possible, CGP via ctDNA assay can be considered. (NCCN Vaginal Cancer Guidelines, v2.2025)

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 September 5, 2024)

Laboratories that perform genetic tests are regulated under the Clinical Laboratory Improvement Amendments (CLIA) Act of 1988. More information is available at: https://www.fda.gov/medicaldevices/deviceregulationandguidance/ivdregulatoryassistance/ucm124105.htm. (Accessed September 5, 2024)

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Policy History/Revision Information

Date	Summary of Changes
04/01/2026	• Retired policy; Louisiana plan membership disenrolled on Apr. 1, 2026
06/01/2025	**Title Change** • Previously titled *Molecular Oncology Companion Diagnostic Testing (for Louisiana Only)* **Coverage Rationale** ***Additional Non State-Specific Criteria*** • Revised coverage guidelines to indicate: ○ Companion Diagnostic Tests are considered proven and medically necessary when the oncology 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 and all of the following criteria are met: ▪ The Companion Diagnostic Test must align with the drug, FDA approved indication, and appropriate tissue/specimen in the FDA List of Cleared or Approved Companion Diagnostic Devices; and ▪ The use of the Companion Diagnostic Test must be consistent with the label for the Companion Diagnostic-associated drug indicated by requesting provider ○ Repeat Companion Diagnostic Testing on a new tissue or Liquid Biopsy specimen for the purpose of assisting with therapy selection is considered proven and medically necessary up to three times annually when the criteria above for Companion Diagnostic Tests are met and one of the following: ▪ The individual is experiencing disease recurrence; or ▪ The individual's cancer has progressed or did not respond to the most recent systemic therapy ○ Concurrent tissue-based and Liquid Biopsy Companion Diagnostic Testing (ordered within 30 days of each other) is considered proven and medically necessary for the following cancer types when the criteria above for Companion Diagnostic Tests are met: ▪ Advanced or metastatic (stage IV) breast cancer ▪ Advanced or metastatic (stage IV) non-small cell lung cancer ○ If no cancer/diagnostic test match is found on the U.S. Food and Drug Administration (FDA) List of Cleared or Approved Companion Diagnostic Devices, refer to the following Medical Policies: ▪ *Molecular Oncology Testing for Solid Tumor Cancer Diagnosis, Prognosis, and Treatment Decisions (for Louisiana Only)* ▪ *Molecular Oncology Testing for Hematologic Cancer Diagnosis, Prognosis, and Treatment Decisions (for Louisiana Only)* **Definitions** • Updated definition of "Comprehensive Genomic Profiling (CGP)" **Applicable Codes** • Added notation to indicate CPT code 0473U is not on the State of Louisiana Medicaid Fee Schedule and therefore may not be covered by the State of Louisiana Medicaid Program

Date	Summary of Changes
	Supporting Information • Updated *Description of Services*, *Clinical Evidence*, and *References* sections to reflect the most current information • Archived previous policy version CS373LA.C

Instructions for Use

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

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