Circulating Tumor DNA and Circulating Tumor Cells for Cancer Management (Liquid Biopsies)

Medical Policy #: 02.04.16
Original Effective Date: March 2008
Reviewed: September 2022
Revised: September 2022

DESCRIPTION

Note: This policy does not address circulating tumor DNA for the management of non-small cell lung cancer, see medical policy 02.04.78 Molecular Analysis (Including Liquid Biopsy) for Targeted Therapy or Immunotherapy of Non-Small Cell Lung Cancer

Liquid Biopsy

Liquid biopsy refers to the analysis of circulating tumor (ctDNA) or circulating tumor cells (CTCs) as methods of noninvasively characterizing tumors and tumor genome from the peripheral blood.

Circulating Tumor DNA
Normal and tumor cells release small fragments of DNA into the blood, which is referred to as cell-free DNA. Cell-free DNA from nonmalignant cells is released by apoptosis. Most cell-free tumor DNA is derived from apoptotic and/or necrotic tumor cells, either from the primary tumor, metastases, or CTCs. Unlike apoptosis, necrosis is considered a pathologic process and generates larger DNA fragments due to incomplete and random digestion of genomic DNA. The length or integrity of the circulating DNA can potentially distinguish between apoptotic and necrotic origin. Circulating tumor DNA can be used for genomic characterization of the tumor.

Circulating Tumor Cells
Intact circulating tumor cells (CTCs) are released from a primary tumor and/or a metastatic site into the bloodstream. The half-life of a CTC in the bloodstream is short (1 to 2 hours), and CTCs are cleared through extravasation into secondary organs. Most assays detect CTCs through the use of surface epithelial markers such as epithelial cell adhesion molecules (EpCAM) and cytokeratins. The primary reason for detecting CTCs is prognostic, through quantification of circulating levels.

Detecting Circulating Tumor DNA and Circulating Tumor Cells
Detection of ctDNA is challenging because ctDNA is diluted by nonmalignant circulating DNA and usually represents a small fraction (<1%) of total cell-free DNA. Therefore, more sensitive methods than the standard sequencing approaches (e.g., Sanger sequencing) are needed.

Highly sensitive and specific methods have been developed to detect ctDNA, for both single nucleotide variants (e.g., BEAMing [which combines emulsion polymerase chain reaction with magnetic beads and flow cytometry] and digital polymerase chain reaction) and copy-number variants. Digital genomic technologies allow for enumeration of rare variants in complex mixtures of DNA.

Approaches to detecting ctDNA can be considered targeted, which includes the analysis of known genetic mutations from the primary tumor in a small set of frequently occurring driver mutations, which can impact therapy decisions, or untargeted without knowledge of specific variants present in the primary tumor, and include array comparative genomic hybridization, next-generation sequencing, and whole exome and genome sequencing.

Circulating tumor cell assays usually start with an enrichment step that increases the concentration of CTCs, either by biologic properties (expression of protein markers) or physical properties (size, density, electric charge). Circulating tumor cells can then be detected using immunologic, molecular, or functional assays.[1,]

The below table summarizes some commercially available tests, and this list may not be comprehensive:

Liquid Biopsy/Circulating Tumor DNA (ctDNA) and Circulating Tumor Cells (CTCs) Tests

Test	Manufacturer	Type of Liquid Biopsy
AR-V7 Prostate Cancer: Detection of androgen receptor variant-7 (AR-V7) transcripts by RT-PCR following selection of circulating tumor cells from patients with metastatic prostate cancer.	Johns Hopkins Medical Institutions – Pathology Laboratory	CTC
CancerIntercept: Breast, ovarian, lung, colorectal, melanoma, head and neck, pancreatic, thyroid, prostate, stomach.	Pathway Genomics	ctDNA
CellMax-First Sight CRC Colorectal Cancer Early Detection Test: used as a screening test for colorectal cancer. Detects pre-cancer and early stage cancer	CellMax Life	CTC
CellMax-LBx Liquid Biopsy: Diagnosed solid tumor for targeted therapy selection	CellMax Life	CTC plus ctDNA
CellMax-PanCa Monitoring Test : Diagnosed solid tumor for treatment effectiveness and early recurrence	CellMax Life	CTC
CellMax-Prostate Cancer Test: To reduce unnecessary biopsies in PSA gray zone patients.	CellMax Life	CTC
Cellsearch Circulating Multiple Myeloma Cell (CMMC) Test: Aids in the monitoring of patients with multiple myeloma to assess the individual's prognosis and is predictive of progression-free survival and overall survival.	Menarini Silicon Biosystems	CTC

CellSearch HER2 Ciruclating Tumor Cell (CTC-HER2) Test: Aids in the monitoring of patients with metastatic breast cancer to assess the individual's prognosis and is predictive of progression-free survival and overall survival.	Menarini Silicon Biosystems	CTC
CellSearch System: Aids in the monitoring of patients with metastatic breast, prostate or colorectal cancer and allows assessment of patient prognosis and is predictive of progression-free survival and overall survival. Informs clinical decision making.	Janssen Diagnostics formerly Veridex	CTC
Circulogene Liquid Biopsy Test: Provides information on current FDA-approved treatment options for the tumor DNA identified. Doctor can monitor tumor responsiveness and adjust treatment protocols.	Theranostics	ctDNA
ClearID Biomarker Expression Assays: For PD-L1 and HER2. **Note:** ClearID test results are summarized in an actionable genomic report containing clinical interpretations of the identified biomarkers and variants, their associations with drugs, related clinical trials, and experimental therapies that can help guide physicians, oncologists, and pathologists in making treatment decisions	Cynvenio	CTC plus ctDNA

ClearID Breast Cancer: Optimized for breast cancer, also used for stomach, skin and prostate cancer. **Note:** ClearID test results are summarized in an actionable genomic report containing clinical interpretations of the identified biomarkers and variants, their associations with drugs, related clinical trials, and experimental therapies that can help guide physicians, oncologists, and pathologists in making treatment decisions	Cynvenio	CTC plus ctDNA
ClearID Lung Cancer: Optimized for non-small cell lung cancer, also used for head and neck cancers, and other thoracic cancers. **Note:** ClearID test results are summarized in an actionable genomic report containing clinical interpretations of the identified biomarkers and variants, their associations with drugs, related clinical trials, and experimental therapies that can help guide physicians, oncologists, and pathologists in making treatment decisions	Cynvenio	CTC plus ctDNA
ClearID Solid Tumor Panel: Optimized for colon and bladder cancer, also used for other solid tumor cancers. **Note:** ClearID test results are summarized in an actionable genomic report containing	Cynvenio	CTC plus ctDNA

clinical interpretations of the identified biomarkers and variants, their associations with drugs, related clinical trials, and experimental therapies that can help guide physicians, oncologists, and pathologists in making treatment decisions.		
Colorectal Cancer Profile: Biocept's liquid biopsy technology allows clinicians to non-invasively evaluate key biomarkers from a simple blood draw, providing information that can be used to guide therapeutic decisions. For colorectal cancers, treatment with EGFR inhibitors can lead to outgrowth of tumor subclones that contain RAS or RAF mutations.[2] Monitoring the molecular evolution of a patient's tumor via serial liquid biopsies therefore provides critical information about tumor burden (e.g., the effectiveness of treatment and the onset of disease progression), as well as the mechanisms of acquired resistance. This information can then be used to rationally adjust treatment strategy.	Biocept	ctDNA
Colvera: Identifies the presence of two methylated genes, BCAT1 and IKZF1, when present show a high concordance of colorectal cancer recurrence.	Clinical Genomics	ctDNA
FoundationOne Liquid CDx:	Foundation Medicine	ctDNA

Analyzes 324 genes, plus it reports blood tumor mutational burden (bTMB), microsatellite instability (MSI) and tumor fraction values. Results can help guide therapy selection and identify clinical trial options for patients with solid tumors.		
Guardant360: For advanced solid tumors, does not predict chemotherapy response but provides information on the genomic alterations known to respond to specific targeted therapies to the doctor the opportunity to tailor treatment to the individual cancer. Point Mutations (SNVs) (73 genes) Indels (23 genes) Amplifications (18 genes) Fusions (6 genes) **Note:** Gauradant360 version updated to Guardant360 CDx	Guardant Health	ctDNA
Guardant360 CDx and Guardant360 Lab Developed Test (LDT): Is a qualitative next generation sequencing-based in virto diagnostic device that uses targeted high throughput hybridization-based capture technology for detection of single nucleotide variants (SNVs), insertions and deletions (indels) in 55 genes, copy number amplifications (CNAs) in 2 genes and fusions in 4 genes.	Guardant Health	ctDNA

<table>
<tr>
<td>Guradant360 CDx utilizes circulating cell-free DNA (cfDNA) from plasma of peripheral whole blood collected in Streck Cell-Free DNA Blood Collection Tubes (BCTs).

The test in intended to be used as a companion diagnostic to identify non-small cell lung cancer (NSCLC) patients who may benefit from treatment with targeted therapy in accordance with approved therapeutic product labeling:

<table><tr><th>Biomarker</th><th>Therapy</th></tr><tr><td>EGFR exon 19 deletions, L858R and T790M</td><td>Tagrisson (Osimertinib)</td></tr></table>
Gurandant360 CDx was FDA approved August 2020</td>
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<td>Guardant Reveal: Detects residual and recurrent disease in early stage cancer patients with colorectal cancer to identify patients who may benefit from adjuvant therapy.</td>
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<td>IVDiagnostics: Used for patients with metastatic advanced staged cancers: breast, lung, ovarian, prostate, colorectal, kidney, melanoma, and pancreatic. Detect and monitor for circulating tumor cells that can cause metastases to rapidly detect and monitor</td>
<td>Velox</td>
<td>CTC</td>
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for these cells to inform therapeutic approaches.		
LiquidGx: Solid tumor therapies, monitoring for drug resistance markers.	Admera Health	ctDNA
miR Sentinel™ Prostate Cancer Test: Uses precise biologic information interrogation techniques to categorize patients into one of four groups: no molecular evidence of prostate cancer, low-risk cancer, intermediate-risk cancer or high-risk cancer which gives the physicians the ability to determine which patients needs treatment and which can be sasfely monitored.	miR Scientific, LLC,	Non-invasive liquid biopsy urine test
NeoLAB Solid Tumor Liquid Biopsy: Is a next generation sequencing (NGS) assay for pan-cancer (bladder, brain, breast, cervical, colorectal, stomach and head and neck) that gives a comprehensive, single order testing solution for solid tumors. It includes 44 genes involved in solid tumor development and progression.	NeoGenomics Laboratories	ctDNA
OncoBEAM for Colorectal Cancer: To assist in treatment decisions for metastatic colorectal cancer.	Sysmex Inostics	ctDNA
OncoBEAM for Melanoma: To assist in treatment decisions for melanoma.	Sysmex Inostics	ctDNA
Oncotype DX AR-V7 Nucleus Detect: Is intended for use in patients with metastatic castration-resistant prostate cancer (mCRPC) who are considering androgen	Genomic Health, Inc.	CTC

receptor signaling inhibitors (e.g. abiraterone, enzalutamide). The tests identifies the presence of AR-V7 protein in the nucleus of circulating tumor cells (CTCs) in the blood to inform clinical decision-making. Eligibility Criteria: have confirmed mCRPC; have received and failed AR-targeted therapy; and are considering additional AR-targeted therapies The results are reported as either AR-V7+ (positive) or AR-V7- (negative)		
PlasmaSelect 64: Multiple cancer types, provides clinical explanation of all reported alterations, including FDA approved therapies, clinical trials and published literature.	Personal Genome Diagnostics	ctDNA
Signatera for Bladder, Breast, and Colon: is a custom-built circulating tumor DNA (ctDNA) test for treatment monitoring and molecular residual disease (MRD) assessment in patients previously diagnosed with cancer. It is not intended to match patients with any particular therapy. Rather, it is intended to detect and quantify how much cancer is left in the body, to detect recurrence earlier, and to help optimize treatment decisions.	Natera, Inc	ctDNA
Target Selector: For breast, colorectal, gastric, prostate,	Biocept	ctDNA

lung, and melanoma to assist the doctor in understanding the status of the patient's disease to make decisions about current and future therapy.		
Tempus xF Liquid Biopsy Panel of 105 Genes: Designed to provide clinical decision support for solid tumors, xF is an alternative to tissue-based biopsies in identifying biomarkers, detecting resistant mutations, monitoring response to treatment or disease progression, and spotting early recurrence in real time. The assay is capable of detecting mutations in two variant classes in 105 genes, including: Single Nucleotide Variants (SNVs) and insertions and deletions (indels), as well as Copy Number Amplifications (CNAs) in 6 genes, Copy Number Deletions (CNDs) in 2 genes, and gene rearrangements (translocations) in 7 genes spanning ~0.3 Mb of genomic space. Microsatellite Instability High (MSI-H) status is also reported when detected. Not Indicated For: • Hematologic malignancies • Early stage (stage I/II) cancers • Brain cancers • Sarcomas	Tempus	ctDNA

PIK3CA Variant Analysis via ctDNA		
therascreen® PIK3CA RGQ PCR Kit: Is used to detect 11 mutations in the PIK3CA gene to determine if a patient with advanced or metastatic breast cancer might be a candidate for PIQRAY treatment.	QIAGEN	ctDNA
PIK3CA Mutation CDx: Is an FDA-approved qualitative companion diagnostic assay performed on cell-free circulating tumor DNA extracted from the peripheral blood plasma of certain breast cancer patients to detect 10 mutations in exons 7, 9, and 20 of the PIK3CA gene. Plasma testing is appropriate when no primary or metastatic breast tumor tissue is available, or the only available tissue is decalcified and therefore unsuitable for molecular testing. Tissue is the recommended specimen type in all other cases. This test is intended to identify PIK3CA mutations in patients with advanced hormone receptor-positive, HER2-negative (HR+/HER2-) breast cancer who may be candidates for therapy with the PI3K alpha-specific inhibitor PIQRAY® (alpelisib).	NeoGenomics Laboratories	ctDNA

Selecting Treatment in Advanced Cancer

Clinical Context and Test Purpose
Treatment selection is informed by tumor type, grade, stage, patient performance status, prior treatments, and the molecular characteristics of the tumor such as the presence of driver mutations. One purpose of the liquid biopsy testing of individuals who have

advanced cancer is to inform a decision regarding treatment selection (e.g., whether to select a targeted a treatment or standard treatment). Individuals have traditionally been tested for driver mutations using samples from tissue biopsies.

Populations
The target population of individuals with advanced cancers for whom the selection of treatment depends on the molecular characterization of the tumor(s).

Interventions
The test being considered is liquid biopsy using either circulating tumor DNA (ctDNA) or circulating tumor cells (CTCs) testing to determine treatment selection. Both targeted polymerase chain reaction (PCR) based assays and broad next generation sequencing (NGS) based approaches are available. Individuals with negative liquid biopsy results should be reflexed to tumor biopsy testing if they are able to undergo tissue biopsy.

Comparators
For individuals who can undergo a biopsy, molecular characterization of the tumor is performed using standard tissue biopsy samples. Patients unable to undergo a biopsy generally receive standard therapy.

The standard of care (SOC) for treatment selection in advanced solid tumors is biomarker analysis of tissue samples obtained by tissue biopsy. Although tumor testing has been primarily focused on use of formalin-fixed paraffin-embedded (FFPE) tissues, increasingly, laboratories accept other specimen types, notably cytopathology preparations not processed by FFPE methods. Although testing on cell blocks is not included in the FDA approval for multiple companion diagnostic assays, testing on these specimen types is highly recommended when it is the only or best material.

While tissue biopsy is required to verify a cancer diagnosis and determine histology, there is often insufficient tissue for genotyping with expert centers reporting rates up to 25%, especially when a gene-by-gene sequential testing approach is utilized. Once tissue is exhausted options include a repeat biopsy or more often treating the patient empirically with standard chemotherapy when the patient may have benefited from targeted therapy.

There are various molecular testing methods that may be used to assess for the different genomic biomarkers:

- Next-generation sequencing (NGS) is used in clinical laboratories. Not all types of alterations are detected by individual NGS assays or combination(s) of assays.
- It is recommended at this time that when feasible, testing be performed via a broad, panel-based approach, most typically performed by next generation sequencing (NGS). For patients who, in broad panel testing don't have identifiable driver oncogenes (especially in never smokers), consider RNA-based NGS if not already performed, to maximize detection of fusion events.

- Real-time polymerase chain reaction (PCR) can be used in a highly targeted fashion (specific mutations targeted). When this technology is deployed, only those specific alterations that are targeted by the assay are assessed.
- Sanger sequencing requires the greatest degree of tumor enrichment. Unmodified Sanger sequencing is not appropriate for detection of mutations in tumor samples with less than 25% to 30% tumor after enrichment is not appropriate for assays in which identification of subclonal events (e.g., resistance mutations) is important. If Sanger sequencing is utilized, tumor enrichment methodologies are nearly always recommended.
- Other methodologies may be utilized, including multiplex approaches not listed above (i.e SNaPshot, MassARRAY).

Outcomes
Liquid biopsies are easier to obtain and less invasive than tissue biopsies. True-positive liquid biopsy test results lead to the initiation of appropriate treatment (e.g., targeted therapy) without a tissue biopsy. False-positive liquid biopsy test results lead to the initiation of inappropriate therapy, which could shorten progression-free survival.

In patients able to undergo a tissue biopsy, negative liquid biopsies reflex to tissue testing. In patients unable to undergo a tissue biopsy, a negative liquid biopsy result would not change empirical treatment. Therefore, health outcomes related to negative test results do not differ between liquid biopsy and tissue biopsy.

The timing of interest for survival outcomes varies by type of cancer.

Review of Evidence

Clinically Valid

Circulating Tumor DNA (ctDNA)
A test must detect the presence or absence of a condition, the risk of developing a condition in the future, or treatment response (beneficial or adverse).

The clinical validity of each commercially available test must be established independently.

Clinically Useful
A test is clinically useful if the use of the results informs management decisions that improve the net health outcome of care. The net health outcome can be improved if patients receive correct therapy, or more effective therapy, or avoid unnecessary therapy or testing.

Direct evidence of clinical utility is provided by studies that have compared health outcomes for patients managed with and without the test. The preferred evidence would be from randomized controlled trials (RCT).

Much of the literature to date on the use of ctDNA to guide treatment selection is for non-small cell lung cancer, which is addressed in medical policy 0*2.04.78 Molecular Analysis (Including Liquid Biopsy) for Targeted Therapy or Immunotherapy of Non-Small Cell Lung Cancer* and is not discussed here.

Merker et. al (2018) the American Society of Clinical Oncology (ASCO) and College of American Pathologists jointly convened a panel to review the current evidence on the use of circulating tumor DNA (ctDNA) in patients with cancer. The literature search identified 1,338 references to which an additional 31 references were supplied by the expert panel. There were 77 articles selected for inclusion. Their analysis on the evidence on the use of ctDNA states the following: Some ctDNA assays have demonstrated clinical validity and utility with certain types of advanced cancer; however, there is insufficient evidence of clinical validity and utility for the majority of ctDNA assays in advanced cancer. Evidence shows discordance between the results of ctDNA assays and genotyping tumor specimens and supports tumor tissue genotyping to confirm undetected results from ctDNA tests. There is no evidence of clinical utility and little evidence of clinical validity of ctDNA assays in early-stage cancer, treatment monitoring, or residual disease detection. There is no evidence of clinical validity and clinical utility to suggest that ctDNA assays are useful for cancer screening, outside of a clinical trial. Given the rapid pace of research, re-evaluation of the literature will shortly be required, along with the development of tools and guidance for clinical practice.

Since the end date of the searches conducted by Merkel et.al. (2018), 4 observational studies of the clinical validity of FoundationOne Liquid CDx (formerly FoundationACT/FoundationOne Liquid) have been published (Table 1). All four studies compared liquid biopsy to tissue biopsy with FoundationOne Liquid CDx comprehensive Genomic testing. Test characteristics are shown in Table 2. Relevance, design and conduct limitation of these studies are summarized in Tables 3 and 4.

Table. 1 Study Characteristics of the Clinical Validity of FoundationOne Liquid CDx

Study	Study Population	Design	Reference Standard	Timing of Reference and Index Tests	Blinding of Assessor
Clark et. al. (2018)	Patients with advanced cancer	Retrospective (tissue) and prospective (liquid biopsy)	Tissue biopsy (FoundationOne)	0 to 60 days	Not stated
Zhou et. al. (2018)	Patients with locally advanced or metastatic solid tumors	Retrospective	Tissue biopsy (FoundationOne)	Not reported; only considered patient with no intervening treatment	Not stated

				between liquid and tissue biopsy	
Chung et. al. (2017)	Women with estrogen receptor-positive breast cancer	Retrospective	Tissue biopsy (FoundationOne)	0 to 60 days	No stated
Kim et. al. (2017)	Woman with measurable, inoperable, locally advanced or metastatic TNBC previously untreated with systemic therapy	Patients were enrolled in a Phase II RCT of Ipataserib plus paclitaxel versus placebo plus paclitaxel	Tissue biopsy (FoundationOne)	Not reported	Not stated

RCT: randomized controlled trials; TNBC: triple-negative breast cancer

Table 2. Clinical Validity of FoundationOne Liquid CDx

Study	Initial N	Final N	PPA	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
Clark et. al. 2018	Not reported	36	75%				
Base substitutions/ Indels				82.7% (69.7-91.8)	97.5% (95.9-98.5)	72.9% (59.7-83.6)	98.6% (97.3-99.4)
Rearrangements				100% (15.8-100)	99.1% (94.3-100)	66.7% (9.4-99.2)	100% (96.5-100)
Amplifications				38.5% (13.9-68.4)	100% (98.5-100)	100% 47.8-100)	96.8% (93.6-98.6)
Zhou et. al, (2018)	Not reported	42	82%				
Base substitutions				77.2% (66.4-85.9)	96.0% (94.6-97.1)	59.2% (49.1-68.8)	98.3% (97.3-99.0)
Insertion/deletions				7.1% 0.9-23.5)	98.2% (95.5-99.5)	33.3% (4.3-77.7)	89.4% (84.9-93)
Amplifications				23.7% (11.4-40.2)	99.8% (98.8-100)	90.0% (53.2-1000	94.1% (91.7-96)

Rearrangements or fusions				100.0% (39.8-100)	97.6% (93.9-99.3)	50.0% 15.7-84.3)	100% (97.7-100)
Chung et. al. (2017							
Short variants	Not reported	14	89%	89.5% (66.9-98.7)	92.4% (84.0-97.3)	73.9% 51.6-89.8)	97.3% (90.2.99.8)
Amplifications	Not reported	14	27%	27.3% (6.0-61)	100% (95.1-100)	100% 29.2-100)	90.1% (81.5-95.6)
Kim et. al. (2017)							
PIK3CA and AKT1	Not reported	72	100%	100% (93.4-100)	100% 81.5-100)	100% (47.8-100)	96.8% (93.6-98.6)

PPA: positive percent agreement; PPV: positive predictive value; NPV: negative predictive value

Table 3. Relevance, Design and Limitations of Clinical Validity Studies of FoundationOne Liquid CDx

Study	Population	Intervention	Comparator	Outcomes	Duration of Follow-Up
Clark et. al. (2018)	Patients with advanced cancer	Earlier version of test used FoundationAct	FoundationOne tissue biopsy	Futher tumor type specific studies are warranted to understand the performance of genomic profiling of ctDNAin the contxt of each cancer type	Follow-up duration not sufficient with respect to natural history of disease (true-positive, true-negative, false-positives, false-negatives cannot be determined)
Zhou et.al.	Patients with locally advanced or metstatic solid tumors (multiple cancer types)	Earlier version of test used FoundationAct	FoundationOne tissue biopsy	Majority of the cases in this study (73%) was NSCLC, further studies are needed to consider the impact of different molecular subtypes and cancer types on	Follow-up duration not sufficient with respect to natural history of disease (true-positive, true-

				tumor metabolic activity that might affect the production of plasma ctDNA	negative, false-positives, false-negatives cannot be determined)
Chung et. al. (2017	Women with estrogen receptor-positive breast cancer	Earlier version of test used FoundationAct	FoundationOne tissue biopsy	Genomic alterations (GA) relevant to relapsed/metastatic breast cancer management were identified, including diverse ESR1 Gas. Genomic profiling of ctDNA demonstrated sensitive detection of mutations found in tissue. Detection of amplifications we associated with ctDNA fraction. Genomic profiling of ctDNA may provide possible alternative approach to tissue-based genomic testing for patients with estrogen-receptor-positive metastatic breast cancer	Follow-up duration not sufficient with respect to natural history of disease (true-positive, true-negative, false-positives, false-negatives cannot be determined)
Kim et. al. (2017)	Women with measurable, inoperable locally advanced or metastatic TNBC previously untreated with	Earlier version of test used FoundationAct	FoundationOne tissue biopsy	These results highlight a potential role of ctDNA in identifying genetic markers associated with improved treatment outcomes	Follow-up duration not sufficient with respect to natural history of disease (true-positive, true-negative,

	systemic therapy				false-positives, false-negatives cannot be determined)

Table 4. Study Design and Limitations of Clinical Validity Studies of FoundationOne Liquid

Study	Selection	Blinding	Delivery of Test	Data Completeness
Clark et. al. (2018)	Selection not random or consecutive (e.g., convenience)	Not blinded to results of reference or other comparator tests	Timing of delivery of index or reference test not described	Inadequate description of indeterminate and missing samples
Zhou et. al. (2018)	Selection not random or consecutive (e.g., convenience)	Not blinded to results of reference or other comparator tests	Timing of delivery of index or reference test not described	Inadequate description of indeterminate and missing samples
Chung et. al. (2017)	Selection not random or consecutive (e.g., convenience)	Not blinded to results of reference or other comparator tests	Timing of delivery of index or reference test not described	Inadequate description of indeterminate and missing samples
Kim et. al. (2017)	Selection not random or consecutive (e.g., convenience)	Not blinded to results of reference or other comparator tests	Timing of delivery of index or reference test not described	Inadequate description of indeterminate and missing samples

In 2017, Vidal et. al. evaluated the clinical validity of the OncoBEAM CRC colorectal cancer assay in a retrospective-prospective study in two Spanish institutions from June 2009 to August 2016 which included 115 patients with histologically confirmed metastatic colorectal cancer (CRC) that were anti-EGFR treatment naive. Blood samples were collected in all patients before the administration of anti-EGFR treatment. OncoBEAM CRC assay was used to detect RAS mutations in plasma and RAS mutation detection in tissue samples were carried out according to standard of care procedures validated by each hospital. The median time from tumor tissue specimen collection to ctDNA collection was 47.5 days (range 0-1783 days). Of the 115 patients included in the study, 55 (47.8%) and 59 (51.3%) were shown to have RAS mutations in their tumor samples as detected by standard of care RAS tissue testing and as detected in ctDNA by OncoBEAM assay and standard techniques for tissue analysis was 93% (107/115 patients), kappa index 0.844 (95% CI 0.746-0.914). There were several limitations to this study to include the retrospective analysis, longitudinal blood extractions were only carried out in limited number of patients and given the low number of patients with

specific clinic-pathological characteristics the inferences from associations with P-values marginally <0.05% should be cautiously interpreted. While this study was promising clinical validity data needs replicated.

Guardant360 CDx
Guardant360 CDx is a qualitative next generation sequencing-based in vitro diagnostic device that uses targeted high throughput hybridization-based capture technology for detection of single nucleotide variants (SNVs), insertions and deletions (indels) in 55 genes, copy number amplifications (CNAs) in 2 genes and fusions in 4 genes. Guradant360 CDx utilizes circulating cell-free DNA (cfDNA) from plasma of peripheral whole blood collected in Streck Cell-Free DNA Blood Collection Tubes (BCTs).

The test in intended to be used as a companion diagnostic to identify non-small cell lung cancer (NSCLC) patients who may benefit from treatment with targeted therapy in accordance with approved therapeutic product labeling:

Biomarker	Therapy
EGFR exon 19 deletions, L858R and T790M	Tagrisson (Osimertinib)

Liquid biopsy (circulating tumor DNA [ctDNA]) for NSCLC is further addressed in medical policy 02.04.7*8 Molecular Analysis (Including Liquid Biopsy) for Targeted Therapy or Immunotherapy of Non-Small Cell Lung Cancer*

Genetic biomarkers are associated in multiple advanced solid tumors however, the evidence is most developed for genetic biomarkers in non-small cell lung cancer (NSCLC) using circulating tumor DNA (ctDNA) in selecting targeted therapy.
The majority of outcome studies for Guardant360 have been for non-small cell lung cancer (NSCLC) (30). Outcome studies for Guardant360 have also been completed in colorectal cancer (7), breast cancer (5) and gastroesophageal cancer (7), however, these studies are limited in size and design.

Liang et al (2016) performed a retrospective chart review of 100 patients with stage 4 or high-risk stage 3 breast cancer. Of the 100 patients included in this study, 29 had a tissue analysis done during the course of treatment. Only the specific genomic alterations tested in both the cell-free DNA (cfDNA) and tissue DNA were included in this analysis. Of the 29 patients with tissue analysis, 6 had no evidence of disease at the time of cfDNA analysis and were excluded from the comparative analysis of genomic alterations found between cfDNA and tissue DNA. A total of 55 single nucleotide variants (SNVs) and 4 copy number variants (CNVs) were evaluated for both cfDNA and tissue DNA from the 23 remaining patients. The degree of agreement between genomic alterations found in tumor DNA (tDNA) and cfDNA was determined by Cohen's Kappa. Clinical disease

progression was compared to mutant allele frequency using a 2-sided Fisher's exact test. The presence of mutations and mutant allele frequency was correlated with PFS using a Cox proportional hazards model and a log-rank test. The most commonly found genomic alterations were mutations in TP53 and PIK3CA, and amplification of EGFR and ERBB2. PIK3CA mutation and ERBB2 amplification demonstrated robust agreement between tDNA and cfDNA (Cohen's kappa = 0.64 and 0.77, respectively). TP53 mutation and EGFR amplification demonstrated poor agreement between tDNA and cfDNA (Cohen's kappa = 0.18 and 0.33, respectively). The directional changes of TP53 and PIK3CA mutant allele frequency were closely associated with response to therapy (p = 0.002). The investigators stated that the presence of TP53 mutation (p = 0.0004) and PIK3CA mutant allele frequency [p = 0.01, HR 1.074 (95 % CI: 1.018 to 1.134)] was excellent predictors of PFS. The authors concluded that identification of selected cancer-specific genomic alterations from cfDNA may be a non-invasive way to monitor disease progression, predict PFS, and offer targeted therapy. They noted that this study was limited by its small sample size and the inherent nature of retrospective data collection of existing genomic information.

Kim et al (2017) reported on an interim analysis of an open-label prospective, clinical trial of ctDNA in patients with metastatic NSCLC, gastric cancer (GC), and other cancers. The investigators reported that somatic alterations were detected in 59 patients with GC (78%), and 25 patients (33%) had targetable alterations (ERBB2, n = 11;MET, n = 5; FGFR2, n = 3; PIK3CA, n = 6). In NSCLC, 62 patients (85%) had somatic alterations, and 34 (47%) had targetable alterations (EGFR, n = 29; ALK, n = 2; RET, n = 1; ERBB2, n = 2). A subgroup of subjects (10 with GC and 17 with NSCLC) who had tissue for confirmation of ctDNA findings were treated with targeted therapy. The investigators reported that response rate and disease control rate were 67% and 100%, respectively, in GC and 87% and 100%, respectively, in NSCLC. The authors noted that this is the first prospective study to examine the clinical utility of comprehensive ctDNA genomic testing to guide matched therapy selection. The authors stated that, because this study was not randomized, its primary limitation is the potential for selection bias to enroll patients more likely to benefit. In addition, the cohort is heterogeneous, including patients at varying lines of therapy and with various concomitant treatments, which limits conclusions in this interim analysis. Not all patients with targetable alterations could receive matched therapy because of the various requirements of the multiple parallel matched therapy substudy protocols, performance status, or loss to follow-up. The authors stated that the final analysis will help to address the modest sample size of this interim analysis as well as report on progression-free survival. The authors stated that future studies should examine ctDNA guided matched therapy outcomes in more racially diverse cohorts.

Willis, et al. (2019) sought to analytically validate microsatellite instability (MSI) testing using Guardant360 according to established guidelines and clinically validate it using 1,145 cfDNA samples for which tissue MSI status based on standard-of-care tissue testing was available. The landscape of cfDNA-based MSI across solid tumor types was investigated in a cohort of 28,459 clinical plasma samples. Clinical outcomes for 16

patients with cfDNA MSI-H gastric cancer treated with immunotherapy were evaluated. In evaluable patients, cfDNA testing accurately detected 87% (71/82) of tissue MSI-H and 99.5% of tissue microsatellite stable (863/867) for an overall accuracy of 98.4% (934/949) and a positive predictive value of 95% (71/75). Concordance of cfDNA MSI with tissue PCR and next-generation sequencing was significantly higher than IHC. Prevalence of cfDNA MSI for major cancer types was consistent with those reported for tissue. Finally, robust clinical activity of immunotherapy treatment was seen in patients with advanced gastric cancer positive for MSI by cfDNA, with 63% (10/16) of patients achieving complete or partial remission with sustained clinical benefit. Limitations included the small number of subjects for which clinical outcomes were evaluated.

In 2019, Maron et. al. evaluated the role of circulating tumor DNA (ctDNA) utilizing 73-gene plasma-based next generation sequencing (NGS) cell-free circulating tumor DNA (ctDNA-NGS) test in guiding clinical decision making of gastroesophageal adenocarcinoma. Gastroesophageal adenocarcinoma (GEA) has a poor prognosis and few therapeutic options. A large cohort was evaluated (n=2140 tests; 1630 patients) of ctDNA-NGS results (including 369 clinically annotated pts). Patients were assessed for genomic alteration (GA) distribution and correlation with clinicopathologic characteristics and outcomes. Treatment history, tumor site, and disease burden dictated tumor-DNA shedding and consequent ctDNA-NGS maximum somatic variant allele frequency (maxVAF). Patients with locally advanced disease having detectable ctDNA post-operatively experienced inferior median disease-free survival (mDFS) (p=0.03). The genomic landscape was similar but not identical to tissue-NGS, reflecting temporospatial molecular heterogeneity, with some targetable GAs identified at higher frequency via ctDNA-NGS compared to previous primary tumor-NGS cohorts. Patients with known microsatellite instability-high (MSI-High) tumors were robustly detected with ctDNA-NGS. Predictive biomarker assessment was optimized by incorporating tissue-NGS and ctDNA-NGS assessment in a complementary manner. HER2-inhibition demonstrated a profound survival benefit in HER2 amplified patients by ctDNA-NGS and/or tissue-NGS (mOS 26.3 versus 7.4 months (p=0.002)), as did EGFR inhibition in EGFR amplified patients (mOS 21.1 versus 14.4 months (p=0.01)). This study had some limitations. The Global cohort, albeit large, was relatively limited in clinical utility without the granular clinicopathologic characteristics to contextualize the GA distribution landscape. Another inherent limitation when comparing the 73-gene cfDNA-NGS versus 315-gene tissue-NGS panel is the expected discordance resulting from technical and biological differences between these different tests of distinct biological compartments. Technical limitations leading to discordance between tissue and plasma obviously included non-overlapping genes, but also some regions of overlapping genes not sequenced on the ctDNA-NGS panel. Another technical limitation is the recognized inability of ctDNA-NGS to discern large-scale deletions amongst the vast sea of wildtype cfDNA. Despite the relatively large size of the clinically annotated cohort, inherent to low-frequency GAs, was our inability to definitively evaluate the prognostic importance of individual GAs nor the predictive impact of targeting these infrequent events. The authors concluded clinical ctDNA-NGS testing holds promise for GEA – both in the detection of minimal residual disease in early- stage disease and as a serial tumor marker. ctDNA-NGS used in

conjunction with tissue-NGS may be an approach to best identify actionable GAs and resistance mechanisms in order to overcome intrapatient heterogeneity. However, prospective validation of these findings in future studies is necessary for integration into clinical care.

In 2019, Patel et. al. investigated the circulating tumor DNA (ctDNA) in pancreatic cancer using clinical laboratory improvement amendments (CLIA) licensed and College of American Pathologist (CAP) accredited clinical laboratory, Guardant Health, Inc. All tissue DNA analyses in this study were performed by a CLIA-licensed and CAP-accredited laboratory, Foundation Medicine, Inc., the assay interrogated 315 genes. ctDNA was analyzed in 112 patients with PDAC (54–73 genes) and tissue DNA in 66 patients (315 genes) (both clinical-grade next-generation sequencing). Number of alterations, %ctDNA, concordance between ctDNA and tissue DNA, and correlation of ctDNA results with survival were assessed. The most common genes altered in ctDNA were TP53 (46% of patients, N = 51) and KRAS (44%, N = 49). Median number of characterized ctDNA alterations per patient was 1 (range, 0–6), but patients with advanced PDAC had significantly higher numbers of ctDNA alterations than those with surgically resectable disease (median, 2 versus 0.5, P = 0.04). Overall, 75% (70/94) of advanced tumors had ≥ 1 ctDNA alteration. Concordance rate between ctDNA and tissue DNA alterations was 61% for TP53 and 52% for KRAS. Concordance for KRAS alterations between ctDNA and tissue DNA from metastatic sites was significantly higher than between ctDNA and primary tumor DNA (72% vs 39%, P = 0.01). Importantly, higher levels of total %ctDNA were an independent prognostic factor for worse survival (hazard ratio, 4.35; 95% confidence interval, 1.85–10.24 [multivariate, P = 0.001]). A patient with three ctDNA alterations affecting the MEK pathway (GNAS, KRAS, and NF1) attained a response to trametinib monotherapy ongoing at 6 months. This study has several limitations. First, the ctDNA gene panel expanded with time, increasing from 54 to 73 genes. Therefore, a limitation of the study pertains to the fact that the sequencing panels were different and so not all gene sequenced in tissue were sequenced in ctDNA. Nonetheless, our tissue and ctDNA panels allowed the comparison of most of the commonly altered genes in pancreatic cancer using clinical-grade assays frequently utilized in patients. The discrepancy in the frequency of CDKN2A/B loss between ctDNA and tissue (with lower frequency in ctDNA) probably results from the fact that its allelic loss was not captured in older panels of the ctDNA sequencing. Second, not all patients had both ctDNA and tissue DNA tests; therefore, future concordance analysis should be performed with larger numbers of patients. Moreover, further analysis with tissue DNA from both primary tissue and metastatic sites may help inform the issues related to intratumoral heterogeneity (though in many patients with pancreatic cancer, accessing biopsy sites can be challenging or dangerous). Third, analysis of the influence of systemic treatment on ctDNA alterations is not feasible in this study due to the lack of serial ctDNA testing per patient. Finally, additional studies are also needed to determine the impact of matching ctDNA alterations to therapy beyond the eight patients matched in the current investigation.

Colorectal cancer (CRC) is the second most common cause of cancer deaths worldwide: The mortality rate is the fourth highest among men and third highest among women. The early diagnosis and treatment of CRC is necessary for clinical progress that improves patient outcomes. Importantly, early CRC detection can significantly improve the cure rate. Traditional clinical diagnostic methods include serum tumor markers, colonoscopy, imaging, and tissue biopsy. Carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA19-9) are used as serum tumor markers, but these two markers alone do not fully satisfy clinical needs due to their lack of sensitivity and specificity. Tumor biopsies also have clinical shortcomings. Due to substantial trauma and poor patient compliance, it is difficult to obtain repeat biopsies to monitor disease progression. Therefore, circulating tumor DNA (ctDNA) has emerged as a promising diagnostic tool for CRC. Isolating and detecting ctDNA is a significant challenge. First, ctDNA accounts for only a small portion of the total cfDNA in peripheral blood (sometimes <0.01%), which makes it difficult to obtain. In 2020, Bi, et. al. provided a review describing the clinical applications and prospects of ctDNA in colorectal cancer (CRC) diagnosis, monitoring and prognosis. The authors concluded as a potential tool for clinical practice, ctDNA has a promising future. However, there are still several areas of the liquid biopsy technology that require development including the clinical examination method, a standardized detection process, and quantitative standards. Variables that affect the sample quality, including sample collection, transportation, and storage, should be controlled. In addition, it is still difficult to separate specific ctDNA fragments from cfDNA. Selecting the best detection panel is also an ongoing challenge. Although ctDNA fragments are currently enriched based on ctDNA/cfDNA fragment length, more research in this area is needed to perform this is the best practice. Currently, the utility of ctDNA is not limited to quantitative assessment, but also provides information related to mutations, copy number variation, and epigenetics. A large quantity of prospective studies with ctDNA are still needed to prove its clinical utility. There are some lingering questions about how to make clinical decisions when ctDNA indicates a possible recurrence, but imaging does not provide an obvious confirmation during a follow-up, and the patients with ctDNA-positive whether need intensive therapy. Needless to say, key benefits of ctDNA are that it provides better metrics for precision medicine and that it breaks away from the limitations of tumor tissue biopsies. Furthermore, ctDNA enables non-invasive treatment monitoring and can inform prognostic evaluations. Ongoing prospective clinical trials with ctDNA are focused on the diagnosis, surveillance, and prognosis of CRC. With the rapid development of science and technology, liquid biopsies will certainly play a key role in the diagnosis and treatment of CRC.

In 2020, Turner et. al. assessed the accuracy of circulating tumor DNA (ctDNA) testing in advanced breast cancer and the ability of ctDNA testing to select patients for mutation-directed therapy. This was an open label, multicohort, phase 2a, platform trial of ctDNA testing in 18 UK hospitals. Participants were women (aged ≥18 years) with histologically confirmed advanced breast cancer and an Eastern Cooperative Oncology Group performance status 0–2. Patients had completed at least one previous line of treatment for advanced breast cancer or relapsed within 12 months of neoadjuvant or adjuvant chemotherapy. Patients were recruited into four parallel treatment cohorts matched to

mutations identified in ctDNA: cohort A comprised patients with ESR1 mutations (treated with intramuscular extended-dose fulvestrant 500 mg); cohort B comprised patients with HER2 mutations (treated with oral neratinib 240 mg, and if oestrogen receptor-positive with intramuscular standard-dose fulvestrant); cohort C comprised patients with AKT1 mutations and oestrogen receptor-positive cancer (treated with oral capivasertib 400 mg plus intramuscular standard-dose fulvestrant); and cohort D comprised patients with AKT1 mutations and oestrogen receptor-negative cancer or PTEN mutation (treated with oral capivasertib 480 mg). Each cohort had a primary endpoint of confirmed objective response rate. For cohort A, 13 or more responses among 78 evaluable patients were required to infer activity and three or more among 16 were required for cohorts B, C, and D. Recruitment to all cohorts is complete and long-term follow-up is ongoing. This trial is registered with ClinicalTrials.gov, NCT03182634; the European Clinical Trials database, EudraCT2015-003735-36; and the ISRCTN registry, ISRCTN16945804. Between Dec 21, 2016, and April 26, 2019, 1051 patients registered for the study, with ctDNA results available for 1034 patients. Agreement between ctDNA digital PCR and targeted sequencing was 96–99% (n=800, kappa 0·89–0·93). Sensitivity of digital PCR ctDNA testing for mutations identified in tissue sequencing was 93% (95% CI 83–98) overall and 98% (87–100) with contemporaneous biopsies. In all cohorts, combined median follow-up was 14·4 months (IQR 7·0–23·7). Cohorts B and C met or exceeded the target number of responses, with five (25% [95% CI 9–49]) of 20 patients in cohort B and four (22% [6–48]) of 18 patients in cohort C having a response. Cohorts A and D did not reach the target number of responses, with six (8% [95% CI 3–17]) of 74 in cohort A and two (11% [1–33]) of 19 patients in cohort D having a response. The most common grade 3–4 adverse events were raised gamma-glutamyltransferase (13 [16%] of 80 patients; cohort A); diarrhea (four [25%] of 20; cohort B); fatigue (four [22%] of 18; cohort C); and rash (five [26%] of 19; cohort D). 17 serious adverse reactions occurred in 11 patients, and there was one treatment-related death caused by grade 4 dyspnea (in cohort C). The availability and accuracy of ctDNA testing shown in this study compares favorably with tissue-based mutation testing. Nearly all patients (99%) received a result from ctDNA testing, contrasting with previous tumor sequencing studies where results were typically received in only 70–90% of patients. In addition, previous tumor sequencing generally only included patients with disease that could be biopsied, which is not consistent for ctDNA testing. Results were achieved relatively quickly after blood draw compared with results from tissue-based testing. The accuracy of ctDNA testing was similar to that achieved with tissue sequencing. Discordance between ctDNA results was still observed for patients at low allele frequency mutations, suggesting further potential for assay development. ESR1 mutations had lower percent-negative agreement, probably reflecting the subclonality of acquired ESR1 mutations, with ctDNA detecting mutations present in metastatic sites other than the one biopsied. Nevertheless, the degree of sensitivity observed in this study suggests that, within the patient population of advanced disease patients recruited, ctDNA testing could replace tissue-based mutation analysis. However, we note that tissue biopsy will remain important for immunohistochemistry, and for copy number-based assessment. Digital PCR offered similar accuracy to sequencing, with substantial cost efficiency, although this comparison was limited to the specific mutations

analyzed. The academic clinical laboratory doing the digital PCR assay achieved the trial target turnaround time of results within 14 days. A shorter turnaround time could easily be achieved if required in clinical practice, resulting in a cost-efficient method of ctDNA analysis. 533 (51·1%) of 1044 patients who underwent ctDNA testing had a potentially targetable mutation (PIK3CA, ESR1, HER2, AKT1, or PTEN), indicating a potential value for ctDNA testing. Study limitations, inclusion of heavily pretreated patients might reduce activity of the targeted drugs, especially in cohort A, and future ctDNA selection trials might benefit from more restrictive entry criteria. The study was designed to asesss the activity of therapies against specific genomic events, but it did not target PIK3Ca mutations and as a result relatively few of the patients registered to the trial had a response to therapy (17 [1-6%] of 1051 patients).

Current NCCN Guidelines:

- **Breast Cancer Version 4.2022**:
    - For HR-positive/HER2-negative breast cancer, assess for PIK3CA mutations with tumor or liquid biopsy to identify candidates for alpelisib plus fulvestrant. PIK3CA mutation testing can be done on tumor tissue or ctDNA in peripheral blood (liquid biopsy). If liquid biopsy is negative, tumor tissue testing is recommended.
    - The clinical use of Circulating Tumor Cells (CTC) or Circulating DNA (ctDNA) in metastatic breast cancer is not yet included in NCCN Guidelines for Breast Cancer for disease assessment and monitoring.
- **Colon Cancer Version 1.2022** Several multigene assays, immunoscore and circulating tumor DNA (ctDNA) have been developed in hopes of providing prognostic and predictive information to aid in decisions regarding adjuvant therapy in patients with Stage II or III colon cancer. Post-surgical ctDNA has been studied as a marker for an elevated risk of recurrence in stage I-III colon cancer. In summary, the information from these tests can further inform risk of recurrence over the other risk factors, but the panel questions the value added. Furthermore, there is no evidence of predictive value in terms of the potential benefit of chemotherapy to any of the available multigene assays. The panel believes there are insufficient data to recommend the use of multigene assays, immunoscore, or post-surgical ctDNA to estimate risk of recurrence or determine adjuvant therapy. ESMO has released similar recommendations regarding these assays, stating that their role in predicting chemotherapy benefit is uncertain. The NCCN Panel encourages enrollment in clinical trials to help with the generation of additional data on these assays.
- **Esophageal and Esophagogastric Junction Cancers Version 4.2022**: The genomic alterations of solid cancers may be identified by evaluating circulating tumor DNA (ctDNA) in the blood, hence a form of "liquid biopsy." Liquid biopsy is being used more frequently in patients with advanced disease who are unable to have clinical biopsy for disease surveillance and management. The detection/alterations in DNA shed with esophageal and EGJ carcinomas can identify targetable alterations or the evolution of clones with altered treatment response profiles. Therefore, for patients who have metastatic or advanced

esophageal/esophagogastric cancers and are unable to undergo a traditional biopsy, testing using a validated NGS-based comprehensive genomic profiling assay performed in a CLIA – approved laboratory may be considered. A negative result should be interpreted with caution, as this does not exclude the presence of tumor mutations or amplifications.

- **Gastric Cancer Version 2.2022**: The genomic alterations of solid cancers may be identified by evaluating circulating tumor DNA (ctDNA) in the blood, hence a form of "liquid biopsy." Liquid biopsy is being used more frequently in patients with advanced disease who are unable to have clinical biopsy for disease surveillance and management. The detection/alterations in DNA shed with gastric carcinomas can identify targetable alterations or the evolution of clones with altered treatment response profiles. Therefore, for patients who are unable to undergo a traditional biopsy, testing using a validated NGS-based comprehensive genomic profiling assay performed in a CLIA-approve laboratory may be considered, A negative result should be interpreted with caution as this does not exclude the presence of tumor mutations or amplifications.
- **Pancreatic Adenocarcinoma Version 1.2022:**
    - In the locally advanced algorithm, there is the following footnote: Tumor/somatic gene profiling is recommended for patients with locally advanced/metastatic disease who are candidates for anti-cancer therapy to identify uncommon mutations. Consider specifically testing for actionable somatic findings including, but not limited: fusions (ALK, NRG1, NTRK, ROS1) mutations (BRAF, BRCA 1/2, HER2, KRAS, PALB2), and mismatch repair (MMR) deficiency (detected tumor IHC, PCR or NGS). Testing on tumor tissue is preferred; however, cell-free DNA testing can be considered if tumor tissue testing is not feasible.
    - In the metastatic disease algorithm, there is the following footnote: Tumor/somatic gene profiling is recommended for patients with locally advanced/metastatic disease who are candidates for anti-cancer therapy to identify uncommon mutations. Consider specifically testing for actionable somatic findings including, but not limited: fusions (ALK, NRG1, NTRK, ROS1) mutations (BRAF, BRCA 1/2, HER2, KRAS, PALB2), and mismatch repair (MMR) deficiency (detected tumor IHC, PCR or NGS). Testing on tumor tissue is preferred; however, cell-free DNA testing can be considered if tumor tissue testing is not feasible.
    - In the disease progression algorithm, there is the following footnote: In the metastatic disease algorithm there is the following footnote: Tumor/somatic gene profiling is recommended for patients with locally advanced/metastatic disease who are candidates for anti-cancer therapy to identify uncommon mutations. Consider specifically testing for actionable somatic findings including, but not limited: fusions (ALK, NRG1, NTRK, ROS1) mutations (BRAF, BRCA 1/2, HER2, KRAS, PALB2), and mismatch repair (MMR) deficiency (detected tumor IHC, PCR or NGS). Testing on tumor tissue is preferred; however, cell-free DNA testing can be considered if tumor tissue testing is not feasible.

- In the recurrence after resection algorithm, there is the following footnote: Tumor/somatic gene profiling is recommended for patients with locally advanced/metastatic disease who are candidates for anti-cancer therapy to identify uncommon mutations. Consider specifically testing for actionable somatic findings including, but not limited: fusions (ALK, NRG1, NTRK, ROS1) mutations (BRAF, BRCA 1/2, HER2, KRAS, PALB2), and mismatch repair (MMR) deficiency (detected tumor IHC, PCR or NGS). Testing on tumor tissue is preferred; however, cell-free DNA testing can be considered if tumor tissue testing is not feasible.

Summary of Evidence
Genetic biomarkers are associated in multiple advanced solid tumors, however, the Guardant360 evidence is most developed for genetic biomarkers in non-small cell lung cancer (NSCLC) using circulating tumor DNA (ctDNA) in selecting targeted therapy to predict response. The NCCN guideline for NSCLC recommends broad molecular profiling using clinically validated test(s) see medical policy *02.04.78 Molecular Analysis (Including Liquid Biopsy) for Targeted Therapy or Immunotherapy of Non-Small Cell Lung Cancer*

Current NCCN guidelines recommend that circulating tumor DNA (ctDNA) may be performed for the following indications in cancer management:

- **Breast Cancer Version 4.2022**: For HR-positive/HER2-negative breast cancer, assess for PIK3CA mutations with tumor or liquid biopsy to identify candidates for alpelisib plus fulvestrant. PIK3CA mutation testing can be done on tumor tissue or ctDNA in peripheral blood (liquid biopsy). If liquid biopsy is negative, tumor tissue testing is recommended.
- **Esophageal and Esophagogastric Junction Cancers Version 4.2022**: The genomic alterations of solid cancers may be identified by evaluating circulating tumor DNA (ctDNA) in the blood, hence a form of "liquid biopsy." Liquid biopsy is being used more frequently in patients with advanced disease who are unable to have clinical biopsy for disease surveillance and management. The detection/alterations in DNA shed with esophageal and EGJ carcinomas can identify targetable alterations or the evolution of clones with altered treatment response profiles. Therefore, for patients who have metastatic or advanced esophageal/esophagogastric cancers and are unable to undergo a traditional biopsy, testing using a validated NGS-based comprehensive genomic profiling assay performed in a CLIA – approved laboratory may be considered. A negative result should be interpreted with caution, as this does not exclude the presence of tumor mutations or amplifications.
- **Gastric Cancer Version 2.2022**: The genomic alterations of solid cancers may be identified by evaluating circulating tumor DNA (ctDNA) in the blood, hence a form of "liquid biopsy." Liquid biopsy is being used more frequently in patients with advanced disease who are unable to have clinical biopsy for disease surveillance and management. The detection/alterations in DNA shed with gastric carcinomas can identify targetable alterations or the evolution of clones with

altered treatment response profiles. Therefore, for patients who are unable to undergo a traditional biopsy, testing using a validated NGS-based comprehensive genomic profiling assay performed in a CLIA-approve laboratory may be considered, A negative result should be interpreted with caution as this does not exclude the presence of tumor mutations or amplifications.

- **Pancreatic Adenocarcinoma Version 1.2022:**
  - In the locally advanced, metastatic disease, disease progression and recurrence after resection algorithms include the following footnote: Tumor/somatic gene profiling is recommended for patients with locally advanced/metastatic disease who are candidates for anti-cancer therapy to identify uncommon mutations. Consider specifically testing for actionable somatic findings including, but not limited: fusions (ALK, NRG1, NTRK, ROS1) mutations (BRAF, BRCA 1/2, HER2, KRAS, PALB2), and mismatch repair (MMR) deficiency (detected tumor IHC, PCR or NGS). Testing on tumor tissue is preferred; however, cell-free DNA testing can be considered if tumor tissue testing is not feasible.

Based on the current NCCN guidelines indicated above circulating tumor DNA (ctDNA) via comprehensive molecular profiling panel Guardant360 CDx/Guardant LDT and FoundationOne may be considered for the management of metastatic or advanced esophageal and esophagogastric junction cancers, advanced gastric cancer and locally advanced or metastatic pancreatic adenocarcinoma if tumor tissue (biopsy) is not feasible. Based on the current NCCN guidelines comprehensive molecular profiling panel (FoundationOne, Guardant360 CDx/Guardant360 LDT not meeting the criteria above is considered not medically necessary, because the testing on tumor tissue is preferred and liquid biopsy/ circulating tumor DNA (ctDNA) should only be considered when tumor tissue testing is not feasible.

Based on the current NCCN guideline the detection and use of liquid biopsy testing/circulating tumor DNA (ctDNA) for PIK3CA variant analysis may be considered in individuals who have recurrent or stage IV hormone receptor -positive/HER2 negative breast cancer. For all other indications in breast cancer management utilizing liquid biopsy testing/circulating tumor DNA (ctDNA) the evidence is insufficient in determining the effects on net health outcomes.

For individuals who have stage II or III colon cancer who receive ctDNA testing, the evidence includes cohort studies. Relevant outcomes are disease-specific survival, test accuracy and validity, and change in disease status. Several cohort studies have reported an association between positive ctDNA results and risk of recurrence of colon cancer. However, while these studies showed an association between ctDNA results and risk of recurrence, they are limited by their observational design and relatively small numbers of patients. Management decisions were not based on ctDNA test results. There are no controlled studies of management changes made in response to ctDNA test results compared to other risk factors, and no studies showing whether testing improved

outcomes. The evidence is insufficient to determine that the technology results in an improvement in the net health outcome.

Based on the peer reviewed medical literature and current NCCN guidelines regarding the detection of circulating tumor DNA (ctDNA) in the treatment and management of advanced cancers other than metastatic or advanced esophageal and esophagogastric junction cancers, advanced gastric cancer and locally advanced or metastatic pancreatic adenocarcinoma if tumor tissue (biopsy) is not available, the evidence is insufficient. While studies may show promise in clinical validity for ctDNA in advanced solid tumors other than those indications listed above if tumor tissue (biopsy) is not feasible, the published studies reporting clinical outcomes and/or clinical utility are lacking. The uncertainties concerning clinical validity and clinical utility preclude conclusions about whether variant analysis of ctDNA can replace variant analysis of tissue in the management of advanced cancers. The clinical value of the entire panel in advanced solid tumors has not been established for all other cancer indications. The evidence is insufficient to determine the effects of the technology on net health outcomes.

Circulating Tumor Cells (CTCs)

Breast Cancer
Results of several observational studies suggest that the use of CellSearch in metastatic breast cancer patients has prognostic value to assess risk of disease progression. Study results showed that enumerated CTC levels by CellSearch were significantly associated with overall survival outcomes in metastatic breast cancer patients. However, results of an RCT reported that use of CellSearch had no impact on survival outcomes in metastatic breast cancer patients who either maintained first-line treatment or opted to change treatment based on CTC levels. A good quality RCT reported that use of CellSearch had no impact on survival outcomes in metastatic breast cancer patients who were undergoing first-line treatment. Thus, clinical utility of CellSearch to predict and monitor treatment response has not been established. There is a lack of evidence regarding how CellSearch compares with variant analysis of tumor biopsy tissue. It is unclear whether molecular subtypes of breast cancer affect the status of CTC under various treatments. There is also a lack of evidence evaluating the use of CellSearch in early stage or non-metastatic breast cancer, or in screening settings.

Prostate Cancer
Oncotype DX AR-V7 Nucleus Detect (Genomic Health, Inc.) is intended for use in patients with metastatic castration-resistant prostate cancer (mCRPC) who are considering androgen receptor signaling inhibitors (i.e., abiraterone, enzalutamide). This test identifies the presence of AR-V7 protein in the nucleus of circulating tumor cells (CTCs) in the blood to inform clinical decision-making. Not all mCRPC patients respond to androgen receptor (AR) targeted therapies, like abiraterone and enzalutamide. When AR-targeted therapy fails, acquired resistance could be the cause. The AR-V7 Nucleus Detect test identifies patients who will not benefit from AR-targeted therapies, is predictive of improved overall survival with taxanes versus AR-targeted therapies and

provides easy to interpret results reported as either AR-V7+ (positive) or AR-V7- (negative). Patients eligible for this testing include those who have confirmed mCRPC, received and failed AR-targeted therapy (i.e., abiraterone, enzalutamide) and will guide subsequent therapeutic decision making.

In 2016 Schreiber et. al., a critical decision in the management metastatic castration-resistant prostate cancer (mCRPC) is when to administer an androgen receptor signaling (ARS) inhibitor or a taxane. Study was performed to determine if pretherapy nuclear androgen-receptor splice variant 7 (AR-V7) protein expression and localization on circulating tumor cells (CTCs) is a treatment-specific marker for response and outcomes between ARS inhibitors and taxanes. For this cross-sectional study at Memorial Sloan Kettering Cancer Center, 265 men with progressive mCRPC undergoing a change in treatment were considered; 86 were excluded because they were not initiating ARS or taxane therapy; and 18 were excluded for processing time constraints, leaving 161 patients for analysis. Between December 2012 and March 2015, blood was collected and processed from patients with progressive mCRPC immediately prior to new line of systemic therapy. Patients were followed up to 3-years. The main outcomes and measures included prostate specific antigen (PSA) response, time receiving therapy, radiographic progression-free survival (rPFS), and overall survival (OS). Overall, of 193 prospectively collected blood samples from 161 men with mCRPC, 191 were evaluable (128 pre-ARS inhibitor and 63 pretaxane). AR-V7–positive CTCs were found in 34 samples (18%), including 3% of first-line, 18% of secondline, and 31% of third- or greater line samples. Patients whose samples had AR-V7–positive CTCs before ARS inhibition had resistant posttherapy PSA changes (PTPC), shorter rPFS, shorter time on therapy, and shorter OS than those without AR-V7–positive CTCs. Overall, resistant PTPC were seen in 65 of 112 samples (58%) without detectable AR-V7–positive CTCs prior to ARS inhibition. There were statistically significant differences in OS but not in PTPC, time on therapy, or rPFS for patients with or without pretherapy AR-V7–positive CTCs treated with a taxane. A multivariable model adjusting for baseline factors associated with survival showed superior OS with taxanes relative to ARS inhibitors when AR-V7–positive CTCs were detected pretherapy (hazard ratio, 0.24; 95% CI, 0.10-0.57; P=.035).

In 2018, Scher et. al. performed a study to determine whether a validated assay for the nuclear-localized androgen receptor splice variant 7 (AR-V7) protein in circulating tumor cells can determine differential overall survival among patients with metastatic castration-resistant prostate cancer (mCRPC) treated with taxanes versus ARS inhibitors This blinded correlative study conducted from December 31, 2012, to September 1, 2016, included 142 patients with histologically confirmed mCRPC and who were treated at Memorial Sloan Kettering Cancer Center, The Royal Marsden, or the London Health Sciences Centre. Blood samples were obtained prior to administration of ARS inhibitors or taxanes as a second-line or greater systemic therapy for progressing mCRPC. The main outcomes and measures included overall survival (OS) after treatment with an ARS inhibitor or taxane in relation to pretherapy AR-V7 status. Among the 142 patients in the study (mean [SD] age, 69.5 [9.6] years), 70 were designated as high risk by conventional prognostic factors. In this high-risk group, patients positive for AR-V7 who were treated

with taxanes had superior overall survival relative to those treated with ARS inhibitors (median overall survival, 14.3 vs 7.3 months; hazard ratio, 0.62; 95% CI, 0.28-1.39 ; *P* .(25. = Patients negative for AR-V7 who were treated with ARS inhibitors had superior overall survival relative to those treated with taxanes (median overall survival, 19.8 vs 12.8 months; hazard ratio, 1.67; 95% CI, 1.00-2.81; *P*=.05). The authors concluded the validated nuclear-localized AR-V7 assay can be used to select a taxane or ARS inhibitor and provide individual patient benefit.

In 2018, Armstrong et. al. conducted and reported on the PROPHECY trial: multicenter prospective trial of circulating tumor cell (CTC) AR-V7 detection in men with metastatic castration-resistant prostate cancer (MCRPC) receiving abiraterone (A) or enzalutamide (E). The primary endpoint was association of baseline AR-V7 with radiographic/clinical progression free survival (PFS), using the Johns Hopkins modified-AdnaTest CTC AR-V7 mRNA assay and the Epic Sciences CTC nuclear AR-V7 protein assay. Overall survival (OS) and PSA decline were key secondary endpoints. Enrolled 118 men with mCRPC starting A/E; 52% had ≥5 Cellsearch CTCs, 36% had prior A/E. On study therapy was A (n = 56), E (n = 59) or both A/E (n = 3). AR-V7 detection by the JHU AR-V7 assay and the Epic AR-V7 assay were independently associated with worse PFS and OS after adjusting for CTC count and established clinical factors (see below table). Concordance between the two AR-V7 assays was 82%. Epic AR-V7 (+) men had more CTC phenotypic heterogeneity: 63% had Shannon Index > 1.5 vs 14% of AR-V7 (-) men; most CTCs in Epic AR-V7 (+) men were AR-V7 (-). We found genetic alterations of aggressive mCRPC in AR-V7 (+) and AR-V7 (-) men including gain of AR, MYCN, and MYC and loss of PTEN, TP53, and DNA repair enzymes in CTCs and ctDNA. The authors concluded they validated AR-V7 detection as an independent CTC-adjusted negative predictive biomarker of short PFS and OS with A/E treatment in men with mCRPC, identify CTC heterogeneity of AR-V7 expression, and highlight the importance of non-AR-V7 drivers of aggressive disease. Clinical Trial Information: NCT02269982.

Outcome	**AR-V7 (JHU n = 116) (+) n = 28 (24%) / (-) n = 88 (66%)**	**AR-V7 (Epic n = 105) (+) n = 11 (10%) / (-) n = 94 (90%)**
Median PFS (mo)	3.1 / 7.3	3.1 / 6.0
p-value	0.0003	0.007
HR* (95% CI)	2.4 (1.6-3.8)	2.4 (1.3-4.6)
HR˚(95% CI)	2.4 (1.4-3.9)	2.2 (1.0-4.9)
Median OS (mo)	11.5 / 25.5	8.4 / 25.5
HR*(95% CI)	3.9 (2.1- 7.3)	4.5 (2.1-9.8)
HR˚ (95% CI	4.6 (2.3-9.2)	3.6 (1.5-8.6)
≥ 50% confirmed PSA decline	11% / 28%	0% / 26%
Odds Ratio (95% CI)	0.31 (0.09-1.12)	Not estimable

*univariate, ˚adjusted for Cellsearch CTC enumeration, PSA, Alk Phos, Hgb

Summary of Evidence
The use of circulating tumor cells (CTCs) has not been proven to impact meaningful health outcomes for most cancers. There is limited evidence to establish the clinical significance of circulating tumor cells (CTCs) and how identification can improve health outcomes. Studies suggest that the identification of circulating tumor cells (CTCs) may have a role in risk stratification and monitoring responses to treatment. National Comprehensive Cancer Network (NCCN) Prostate Cancer Version 1.2023 states "AR-V7 testing in circulating tumor cells (CTSs) can be considered to help guide selection of therapy in the post-abirterone/enzalutamide metastatic CRPC Setting." Except for testing for the AR-V7 variant in metastatic castrate-resistant prostate cancer (mCRPC) the role of this testing in patient management is not yet known. Larger longitudinal studies with standard techniques in clearly defined populations of patients are needed to establish the role of this testing in all other cancer indications. The evidence is insufficient to determine the effects for the technology on net health outcomes except as indicated above for prostate cancer based on current NCCN guideline recommendations.

Predicting Risk of Relapse/Recurrence

Clinical Context and Test Purpose
Monitoring for relapse after curative therapy in patients with cancer may be performed using imaging methods and clinical examination. Another proposed purpose of liquid biopsy testing in patients who have cancer is to detect and monitor for residual tumor, which could lead to early treatment that would eradicate residual disease and potentially improve outcomes.

Populations
The relevant population of interest are patients who have received curative treatment for cancer.

Interventions
The test being considered is liquid biopsy using ctDNA.

Comparators
Standard monitoring methods for detecting relapse are imaging methods and clinical examination.

Outcomes
The outcome of primary interest is progression-free survival.

The timing of interest for survival outcomes varies by type of cancer.

Clinically Valid

Circulating Tumor DNA (ctDNA)
Merker et. al (2018) identified several proof-of-principle studies demonstrating an association between persistent detection of ctDNA after local therapy and high-risk of relapse. However, current studies are retrospective and have not systematically confirmed that ctDNA is being detected before the metastatic disease has developed. They concluded that the performance characteristics had not been established for any assays.

The clinical validity of each commercially available CTC test must be established independently.

Signatera Assay
Is a circulating tumor DNA (ctDNA) for molecular residual disease (MRD) assessment and recurrence monitoring for patients previously diagnosed with cancer. Signatera assay can be used to detect recurrence earlier while it still may be resectable and reduce false positives.

Colon Cancer
2019 Reinert et. al., investigated the association of circulating tumor DNA (ctDNA) with recurrence using longitudinal data from ultradeep sequencing of plasma cell-free DNA in patients with colorectal cancer (CRC) before and after surgery, during and after adjuvant chemotherapy (ACT), and during surveillance. Outcomes were ctDNA measurement, clinical recurrence, and recurrence-free survival. A total of 130 patients with stages I to III CRC (mean [SD] age, 67.9 [10.1] years; 74 [56.9%] male) were enrolled in the study; 5 patients discontinued participation, leaving 125 patients for analysis. Preoperatively, ctDNA was detectable in 84 of 94 patients (89.4%). After definitive treatment, longitudinal ctDNA analysis identified 14 of 16 relapses (87.5%). At postoperative day 30, ctDNA-positive patients were 7 times more likely to relapse than ctDNA-negative patients (hazard ratio [HR], 7.2; 95% CI, 2.7-19.0; $P < .001$). Similarly, shortly after ACT ctDNA-positive patients were 17 times (HR, 17.5; 95% CI, 5.4-56.5; $P < .001$) more likely to relapse. All 7 patients who were ctDNA positive after ACT experienced relapse. Monitoring during and after ACT indicated that 3 of the 10 ctDNA-positive patients (30.0%) were cleared by ACT. During surveillance after definitive therapy, ctDNA-positive patients were more than 40 times more likely to experience disease recurrence than ctDNA-negative patients (HR, 43.5; 95% CI, 9.8-193.5 $P < .001$). In all multivariate analyses, ctDNA status was independently associated with relapse after adjusting for known clinicopathologic risk factors. Serial ctDNA analyses revealed disease recurrence up to 16.5 months ahead of standard-of-care radiologic imaging (mean, 8.7 months; range, 0.8-16.5 months). Actionable mutations were identified in 81.8% of the ctDNA-positive relapse samples.

Wang et. al. (2019) evaluated whether serial circulating tumor DNA (ctDNA) levels detected disease recurrence earlier, compared with conventional postoperative surveillance, in patients with resected colorectal cancer (CRC). This study included patients (n = 58) with stage I, II, or III CRC who underwent radical surgical resection at 4 Swedish hospitals from February 2, 2007, to May 8, 2013. Eighteen patients received

adjuvant chemotherapy at the discretion of their clinicians, who were blinded to the ctDNA results. Blood samples were collected at 1 month after the surgical procedure and every 3 to 6 months thereafter for ctDNA analysis. Patients were followed up until metachronous metastases were detected, or for a median of 49 months. Data analysis was performed from March 1, 2009, to June 23, 2018. Sensitivity and timing of ctDNA positivity were compared with those of conventional surveillance modalities (computed tomographic scans and serum carcinoembryonic antigen tests) for the detection of disease recurrence. This study included 319 blood samples from 58 patients, with a median (range) age of 69 (47-83) years and 34 males (59%). The recurrence rate among patients with positive ctDNA levels was 77% (10 of 13 patients). Positive ctDNA preceded radiologic and clinical evidence of recurrence by a median of 3 months. Of the 45 patients with negative ctDNA throughout follow-up, none (0%; 95% CI, 0%-7.9%) experienced a relapse, with a median follow-up of 49 months. However, 3 (6%; 95% CI, 1.3%-17%) of the 48 patients without relapse had a positive ctDNA result, which subsequently fell to undetectable levels during follow-up. The authors concluded although these findings need to be validated in a larger, prospective trial, they suggest that ctDNA analysis could complement conventional surveillance strategies as a triage test to stratify patients with resected CRC on the basis of risk of disease recurrence.

Two cohort studies reported an association between positive ctDNA results using the Signatera assay and risk of recurrence of colon cancer. While these studies showed an association between ctDNA results and risk of recurrence, they are limited by their observational design and relatively small numbers of patients with positive results. Management decisions were not based on ctDNA test results. There are no controlled studies of management changes made in response to ctDNA test results compared to other risk factors, and no studies showing whether testing improved outcomes.

For individuals who have stage II or III colon cancer who receive circulating tumor DNA (ctDNA) testing, the evidence includes cohort studies. Relevant outcomes are disease-specific survival, test accuracy and validity, and change in disease status. Two cohort studies reported an association between positive ctDNA results and risk of recurrence of colon cancer. In one study, the recurrence rate among patients with positive ctDNA levels was 77% (10 of 13 patients); no patients with negative ctDNA experienced a relapse over a median followup of 49 months (range 11-70 months). In the other, the recurrence rate at 3 years was 70% in patients with a positive ctDNA test compared to 11.9% of those with a negative ctDNA test. While these studies showed an association between ctDNA results and risk of recurrence, they are limited by their observational design and relatively small numbers of patients with positive results. Management decisions were not based on ctDNA test results.There are no controlled studies of management changes made in response to ctDNA test results compared to other risk factors, and no studies showing whether testing improved outcomes. The evidence is insufficient to determine the effects of the technology on health outcomes.

Colorectal Cancer

In 2013, Deneve et. al. reported on a study related to the capture of viable circulating tumor cells (CTCs) in the liver of colorectal cancer patients (CRC). The incidence and number of circulating tumor cells (CTCs) in the peripheral blood of colorectal cancer patients are lower than in other cancer types, which may point to a particular biology of colorectal cancer affecting CTC detection. They detected CTCs in the peripheral and mesenteric blood of colorectal cancer patients by use of 2 independent technologies on the basis of different biological properties of colon cancer cells. Seventy-five patients diagnosed with localized (M0, n = 60) and metastatic (M1, n = 15) colorectal cancer were included. Peripheral and mesenteric blood samples were collected before tumor resection. We performed CTC enumeration with an EpCAM-independent enrichment method followed by the Epispot assay that detected only viable CK19-releasing CTCs. In parallel, we used the FDA-cleared EpCAM-dependent CellSearch as the reference method. The enumeration of CK19-releasing cells by the CK19-Epispot assay revealed viable CTCs in 27 of 41 (65.9%) and 41 of 74 (55.4%) (P = 0.04) patients in mesenteric and peripheral blood, respectively, whereas CellSearch detected CTCs in 19 of 34 (55.9%) and 20 of 69 (29.0%) (P = 0.0046) patients. In mesenteric blood, medians of 4 (range 0-247) and 2.7 CTCs (range 0-286) were found with Epispot and CellSearch (P = 0.2), respectively, whereas in peripheral blood, Epispot and CellSearch detected a median of 1.2 (range 0-92) and 0 CTCs (range 0-147) (P = 0.002). A considerable portion of viable CTCs detectable by the Epispot assay are trapped in the liver as the first filter organ in CRC patients. The authors concluded, future investigations should focus on defining the best markers of the subpopulation of functional CTCs that are the metastasis initiating cells, defining the role of EpCAM in liver metastases formation, and identifying factors from colon CTCs able to induce the prometastatic microenvironment of the liver.

Bladder Cancer

Rink et. al. (2012) prospectively analyzed the prognostic role and HER2 expression of circulating tumor cells (CTCs) in peripheral blood of patients prior to radical cystectomy with clinically non-metastatic urothelial carcinoma of the bladder (UCB). Blood samples from 100 consecutive UCB patients treated with radical cystectomy (RC) were investigated for the presence (CellSearch system) of CTC and their HER2 expression status (immunohistochemistry). HER2 expression of the corresponding primary tumors and lymph node metastasis were analyzed using fluorescence in situ hybridization. Blood samples were taken preoperatively. Patients underwent RC with lymphadenectomy. Outcomes were assessed according to CTC status. HER2 expression of CTC was compared with that of the corresponding primary tumor and lymph node metastasis. CTC were detected in 23 of 100 patients (23%) with non-metastatic UCB (median: 1; range: 1-100). Presence, number, and HER2 status of CTC were not associated with clinicopathologic features. CTC-positive patients had significantly higher risks of disease recurrence and cancer-specific and overall mortality (p values: ≤ 0.001). After adjusting for effects of standard clinicopathologic features, CTC positivity remained an independent predictor for all end points (hazard ratios: 4.6, 5.2, and 3.5, respectively; p values ≤ 0.003). HER2 was strongly positive in CTC from 3 of 22 patients (14%). There was discordance between HER2 expression on CTC and HER2 gene amplification status

of the primary tumors in 23% of cases but concordance between CTC, primary tumors, and lymph node metastases in all CTC-positive cases (100%). The study was limited by its sample size.

In 2014, Gazzaniga et. al. performed study to investigate whether the presence of circulating tumor cells (CTCs) may improve prognostication in a large population of patients with Stage I bladder cancer who were all candidates for conservative surgery. A prospective single center trial was designed to correlate the presence of CTC to local recurrence and progression of disease in high-risk T1G3 bladder cancer. One hundred two patients were found eligible, all candidate to transurethral resection of the tumor followed by endovesical adjuvant immunotherapy with BCG. Median follow-up was 24.3 months (minimum-maximum: 4-36). The FDA-approved CellSearch System was used to enumerate CTC. Kaplan-Meier methods, log-rank test and multivariable Cox proportional hazard analysis was applied to establish the association of circulating tumor cells with time to first recurrence (TFR) and progression-free survival. CTC were detected in 20% of patients and predicted both decreased TFR (log-rank $p < 0.001$; multivariable adjusted hazard ratio [HR] 2.92 [95% confidence interval: 1.38-6.18], $p = 0.005$), and time to progression (log-rank $p < 0.001$; HR 7.17 [1.89-27.21], $p = 0.004$).

In 2019, Christensen et. al. addressed the prognostic and predictive impact of ultra-deep sequencing of cell-free DNA in patients before and after cystectomy and during chemotherapy. This study included 68 patients with localized advanced bladder cancer. Patient-specific somatic mutations, identified by whole-exome sequencing, were used to assess circulating tumor DNA (ctDNA) by ultra-deep sequencing (median, 105,000×) of plasma DNA. Plasma samples (n = 656) were procured at diagnosis, during chemotherapy, before cystectomy, and during surveillance. Expression profiling was performed for tumor subtype and immune signature analyses. Presence of ctDNA was highly prognostic at diagnosis before chemotherapy (hazard ratio, 29.1; P = .001). After cystectomy, ctDNA analysis correctly identified all patients with metastatic relapse during disease monitoring (100% sensitivity, 98% specificity). A median lead time over radiographic imaging of 96 days was observed. In addition, for high-risk patients (ctDNA positive before or during treatment), the dynamics of ctDNA during chemotherapy was associated with disease recurrence (P = .023), whereas pathologic downstaging was not. Analysis of tumor-centric biomarkers showed that mutational processes (signature 5) were associated with pathologic downstaging (P = .024); however, no significant correlation for tumor subtypes, DNA damage response mutations, and other biomarkers was observed. Our results suggest that ctDNA analysis is better associated with treatment efficacy compared with other available methods and a basis for clinical studies that evaluate early therapeutic interventions.

Liver Cancer

Schulze et. al. (2013) investigated the prognostic relevance of EpCAM-positive circulating tumor cells (CTCs) in patients with HCC. Current imaging technologies do not sufficiently detect micrometastasis and therefore do not allow adequate stratification

of patients with hepatocellular carcinoma (HCC) for curative or systemic therapy. Blood from 78 patients (19 patients in the control cohort and 59 patients with HCC) was tested for CTCs with the CellSearch system. Correlation analysis to overall survival (OS), the Barcelona Clinic Liver Cancer (BCLC) staging system, macroscopic and microscopic vascular invasion and alpha-fetoprotein (AFP) levels were performed. They detected ≥1 CTC in 18/59 HCC patients and in 1/19 patients with cirrhosis or benign hepatic tumor ($p = 0.026$). OS was significantly shorter (460 vs. 746 days) in the CTC-positive cohort ($p = 0.017$). Comparing BCLC stages, significant differences in CTC detection rates were also observed: BCLC stages A 1/9, B 6/31 and C 11/19 ($p = 0.006$). Ten of 18 patients with macroscopic and 10/16 patients with microscopic vascular invasion exhibited positive findings in CTC testing ($p = 0.004$ and $p = 0.006$). The authors concluded; CTC results correlated to AFP (cutoff > 400 ng/mL) levels ($p = 0.050$). The study demonstrates frequent presence of EpCAM-positive CTC in patients with intermediate or advanced HCC and its prognostic value for OS with possible implications for future treatment stratification.

Summary of Evidence
There is no direct evidence that using circulating tumor DNA (ctDNA) or circulating tumor cells (CTCs) to predict the risk of relapse/recurrence improves the net health outcome compared with standard methods. Given the different methodologies available to assess ctDNA and CTCs, the clinical validity of each commercially available test must be established independently, and these data are lacking. The evidence is insufficient to demonstrate test performance for currently available ctDNA and CTCs tests and therefore, no conclusion can be made about the clinical utility through a chain of evidence. Further high quality, well designed, large prospective studies are needed to explore and establish whether individualized therapeutic decisions based on ctDNA would improve net health outcomes. The evidence is insufficient to determine the effects of the technology on net health outcomes.

Circulating Tumor DNA (ctDNA) and Circulating Tumor Cells (CTCs) Screening for Cancer in Asymptomatic Individuals

Clinical Context and Test Purpose
It has been proposed that liquid biopsies could be used to screen asymptomatic patients for early detection of cancer, which could allow for initiating treatment at an early stage, potentially improving outcomes.

Populations
The relevant population of interest are asymptomatic individuals.

Interventions
The test being considered is liquid biopsy using either ctDNA or CTCs.

Examples of commercially available testing:
The Galleri Test can provide two possible results:

- No cancer signal detected means there is no cancer DNA detected in the individual's bloodstream.
- A cancer signal detected suggests the individual may have cancer.

The Galleri can detect more than 50 types of cancer, including the following:

- Anal cancer
- Breast cancer
- Cervical cancer
- Esophageal cancer
- Kidney cancer
- Leukemia
- Liver cancer
- Mesothelioma
- Oral cancer
- Pancreatic cancer
- Stomach cancer
- Uterine cancer

HelioLiver Test
The HelioLiver test is intended for surveillance in individuals diagnosed with liver cirrhosis, to help detect hepatocellular carcinoma (HCC) earlier allowing individuals access to more curative treatment options and improving outcomes overall. The HelioLiver test is a multi-analyte blood testing combining cell-free DNA (cfDNA) methylation patters and protein biomarkers.

Comparators
The following practice is currently being used: standard screening methods.

Outcomes
The outcome of primary interest is overall survival, disease specific survival, and test validity.

The timing of interest for survival outcomes varies by type of care.

Diagnosis of cancer that is not present or would not have become clinically important (false-positives and over-diagnosis) would lead to unnecessary treatment and treatment related morbidity.

Review of Evidence

Clinically Valid
A test must detect the presence or absence of a condition, the risk of developing a condition in the future, or treatment response (beneficial or adverse).

Circulating Tumor DNA (ctDNA)
Merker et. al. (2018) reported there is no evidence of clinical validity for the use of ctDNA in asymptomatic individuals.

Circulating Tumor Cells (CTCs)
Systematic reviews with meta-analyses have evaluated the diagnostic accuracy of CTCs in patients with gastric and bladder/urothelial cancer. Reported sensitivity was low in both cancers (42% and 35%) overall. Sensitivity was lower in patients with early-stage cancer, suggesting that the test would not be useful as an initial screen.

The clinical validity of each commercially available CTC test must be established independently.

Clinically Useful
A test is clinically useful if the use of the results informs management decisions that improve the net health outcomes of care. The net health outcome can be improved if patients receive correct therapy, or more effective therapy, or avoid unnecessary therapy, or avoid unnecessary testing.

Direct evidence of clinical utility is provided by studies that have compared health outcomes for patients managed with and without the test. Because these are intervention studies, the preferred evidence would be from randomized controlled trials (RTCs).

To evaluate the utility of the tests for screening, guidelines would be needed to establish criteria for screening intervals and appropriate follow-up for positive tests. After such guidelines are established, studies demonstrating the liquid biopsy test performance as a cancer screening test would be needed.

Chain of Evidence
Indirect evidence on clinical utility rests on clinical validity. If the evidence is insufficient to demonstrate test performance, no inferences can be made about clinical utility. Also, a chain of evidence requires an evidence-based management pathway. There is not a clear, evidence-based management pathway for the use of ctDNA or CTCs for the screening of asymptomatic patients.

The evidence is insufficient to demonstrate test performance for currently available ctDNA and CTC tests as a screening test for cancer; therefore, no inferences can be made about clinical utility through a chain of evidence.

Summary of Evidence
For individuals who are asymptomatic and at high-risk for cancer who receive testing of ctDNA to screen for cancer, no evidence was identified. Relevant outcomes are overall survival (OS), disease-specific survival, test accuracy, and test validity. Published data on clinical validity and clinical utility are lacking. Based on the 2018 ASCO guideline, there is no evidence of clinical validity and clinical utility to suggest that ctDNA assays are

useful for cancer screening, outside of a clinical trial. The evidence is insufficient to determine the effects of the technology on health outcomes.

For individuals who are asymptomatic and at high-risk for cancer who receive testing of CTCs to screen for cancer, the evidence includes observational studies. Given the breadth of methodologies available to assess CTCs, the clinical validity of each commercially available test must be established independently, and this data is lacking. Published studies reporting clinical outcomes and/or clinical utility are also lacking. The evidence is insufficient to determine the effects of the technology on health outcomes.

Practice Guidelines and Position Statements

American Society of Clinical Oncology (ASCO) and College of American Pathologists

In 2018, the American Society of Clinical Oncology (ASCO) and College of American Pathologists issued a joint review on circulating tumor DNA in patients with cancer which states: Some ctDNA assays have demonstrated clinical validity and utility with certain types of advanced cancer; however, there is insufficient evidence of clinical validity and utility for the majority of ctDNA assays in advanced cancer. Evidence shows discordance between the results of ctDNA assays and genotyping tumor specimens and supports tumor tissue genotyping to confirm undetected results from ctDNA tests. There is no evidence of clinical utility and little evidence of clinical validity of ctDNA assays in early-stage cancer, treatment monitoring, or residual disease detection. There is no evidence of clinical validity and clinical utility to suggest that ctDNA assays are useful for cancer screening, outside of a clinical trial. Given the rapid pace of research, re-evaluation of this literature will shortly be required, along with the development of tools and guidance for clinical practice.

National Comprehensive Care Network (NCCN)

Treatment by Cancer Type	Recommendation
Anal Carcinoma Version 2.2022	The clinical use of circulating tumor cells (CTC) or circulating tumor DNA (ctDNA) in anal carcinoma is not included in this current NCCN guideline.
Bladder Cancer 2.2022	The clinical use of circulating tumor cells (CTC) or circulating tumor DNA (ctDNA) in bladder cancer is not included in this NCCN guideline.
Breast Cancer 4.2022	For HR-positive/HER2-negative breast cancer, assess for PIK3CA mutations with tumor or liquid biopsy to identify candidates for alpelisib plus fulvestrant. PIK3CA mutation testing can be done on tumor tissue or ctDNA in peripheral blood

	(liquid biopsy). If liquid biopsy is negative, tumor tissue testing is recommended. The clinical use of circulating tumor cells or circulating DNA (ctDNA) in metastatic breast disease is not yet included in NCCN Guidelines for Breast Cancer for disease assessment and monitoring.
Cervical Cancer 1.2022	The clinical use of circulating tumor cells (CTC) or circulating tumor DNA (ctDNA) in cervical cancer is not included in this current NCCN guideline.
Colon Cancer 1.2022	The clinical use of circulating tumor cells (CTC) or circulating tumor DNA (ctDNA) in colon cancer is not included in this current NCCN guideline. **Multigene Assays, Immunoscore, and Circulating Tumor DNA (ctDNA)** Several multigene assays have been developed in hopes of providing prognostic and predictive information to aid in decisions regarding adjuvant therapy in patients with Stage II or III colon cancer. Post-surgical ctDNA has been studied as a marker for an elevated risk of recurrence in stage I-III colon cancer. In summary, the information from these tests can further inform risk of recurrence over the other risk factors, but the panel questions the value added. Furthermore, there is no evidence of predictive value in terms of the potential benefit of chemotherapy to any of the available multigene assays. The panel believes there are insufficient data to recommend the use of multigene assays, immunoscore, or post-surgical ctDNA to estimate risk of recurrence or determine adjuvant therapy. ESMO has released similar

	recommendations regarding these assays, stating that their role in predicting chemotherapy benefit is uncertain. The NCCN Panel encourages enrollment in clinical trials to help with the generation of additional data on these assays.
Esophageal and Esophagogastric Junction Cancers Version 4.2022	**Principles of Pathologic Review and Biomarker Testing** **Liquid Biopsy** The genomic alterations of solid cancers may be identified by evaluating circulating tumor DNA (ctDNA) in the blood, hence a form of "liquid biopsy." Liquid biopsy is being used more frequently in patients with advanced disease who are unable to have clinical biopsy for disease surveillance and management. The detection/alterations in DNA shed with esophageal and EGJ carcinomas can identify targetable alterations or the evolution of clones with altered treatment response profiles. Therefore, for patients who have metastatic or advanced esophageal/esophagogastric cancers and are unable to undergo a traditional biopsy, testing using a validated NGS-based comprehensive genomic profiling assay performed in a CLIA – approved laboratory may be considered. A negative result should be interpreted with caution, as this does not exclude the presence of tumor mutations or amplifications.
Gastric Cancers 2.2022	**Principles of Pathologic Review and Biomarker Testing** Liquid Biopsy The genomic alterations of solid cancers may be identified by evaluating circulating tumor DNA (ctDNA) in the blood, hence a form of "liquid biopsy." Liquid biopsy is being used more frequently in patients with advanced disease who are unable to have clinical biopsy for disease surveillance

	and management. The detection/alterations in DNA shed gastric carcinomas can identify targetable alterations or the evolution of clones with altered treatment response profiles. Therefore, for patients who are unable to undergo a traditional biopsy, testing using a validated NGS-based comprehensive genomic profiling assay performed in a CLIA-approve laboratory may be considered, A negative result should be interpreted with caution as this does not exclude the presence of tumor mutations or amplifications.
Hepatobiliary Cancers Version 2.2022	The clinical use of circulating tumor cells (CTC) or circulating tumor DNA (ctDNA) in hepatobiliary cancers is not included in this current NCCN guideline.
Kidney Cancer Version 2.2023	The clinical use of circulating tumor cells (CTC) or circulating tumor DNA (ctDNA) in kidney cancer is not included in this current NCCN guideline.
Melanoma: Cutaneous Version 3.2022	The clinical use of circulating tumor cells (CTC) or circulating tumor DNA (ctDNA) in cutaneous melanoma is not included in this current NCCN guideline.
Ovarian Cancer/Fallopian Tube Cancer/Primary Peritoneal Cancer 2.2022	The clinical use of circulating tumor cells (CTC) or circulating tumor DNA (ctDNA) in Ovarian Cancer Including Fallopian Tube Cancer and Primary Peritoneal Cancer is not included in this current NCCN guideline.
Pancreatic Adenocarcinoma 1.2022	In the locally advanced algorithm, there is the following footnote: Tumor/somatic gene profiling is recommended for patients with locally advanced/metastatic disease who are candidates for anti-cancer therapy to identify uncommon mutations. Consider specifically testing for actionable somatic findings including, but

	not limited: fusions (ALK, NRG1, NTRK, ROS1) mutations (BRAF, BRCA 1/2, HER2, KRAS, PALB2), and mismatch repair (MMR) deficiency (detected tumor IHC, PCR or NGS). **Testing on tumor tissue is preferred; however, cell-free DNA testing can be considered if tumor tissue testing is not feasible.** In the metastatic disease algorithm, there is the following footnote: Tumor/somatic gene profiling is recommended for patients with locally advanced/metastatic disease who are candidates for anti-cancer therapy to identify uncommon mutations. Consider specifically testing for actionable somatic findings including, but not limited: fusions (ALK, NRG1, NTRK, ROS1) mutations (BRAF, BRCA 1/2, HER2, KRAS, PALB2), and mismatch repair (MMR) deficiency (detected tumor IHC, PCR or NGS). **Testing on tumor tissue is preferred; however, cell-free DNA testing can be considered if tumor tissue testing is not feasible.** In the disease progression algorithm, there is the following footnote: In the metastatic disease algorithm there is the following footnote: Tumor/somatic gene profiling is recommended for patients with locally advanced/metastatic disease who are candidates for anti-cancer therapy to identify uncommon mutations. Consider specifically testing for actionable somatic findings including, but not limited: fusions (ALK, NRG1, NTRK, ROS1) mutations (BRAF, BRCA 1/2, HER2, KRAS, PALB2), and mismatch repair (MMR) deficiency (detected tumor IHC, PCR or NGS). **Testing on tumor tissue is preferred; however, cell-free DNA testing can be considered if tumor tissue testing is not feasible.**

	In the recurrence after resection algorithm there is the following footnote: Tumor/somatic gene profiling is recommended for patients with locally advanced/metastatic disease who are candidates for anti-cancer therapy to identify uncommon mutations. Consider specifically testing for actionable somatic findings including, but not limited: fusions (ALK, NRG1, NTRK, ROS1) mutations (BRAF, BRCA 1/2, HER2, KRAS, PALB2), and mismatch repair (MMR) deficiency (detected tumor IHC, PCR or NGS). **Testing on tumor tissue is preferred; however, cell-free DNA testing can be considered if tumor tissue testing is not feasible**
Penile Cancer 2.2022	The clinical use of circulating tumor cells (CTC) to circulating tumor DNA (ctDNA) in penile cancer is not included in this current NCCN guideline.
Prostate Cancer 1.2023	AR-V7 testing in circulating tumor cells (CTSs) can be considered to help guide selection of therapy in the post-abirterone/enzalutamide metastatic CRPC setting. Lack of response of men with metastatic CRPC to abiraterone and enzalutamide was associate with detection of AR-V7 mRNA in CTCs using an RNA-based polymerase chain reaction (PCR) assay. Ar-V7 presence did not preclude clinical benefit from taxane chemotherapies (docetaxel and cabazitaxel). Men with AR-V7 positive CTCs exhibited superior PFS with taxanes compared to novel hormonal therapies (abiraterone and enzalutamib); the two classes of agents resulted in comparable PFS in men with AR-V7 negative CTCs. A confirmatory study used a different CTC assay that detected nuclear-localized AR-V7 protein using immunofluorescence. Men with

	AR-V7 positive CTCs had superior OS with taxanes versus abiraterone or enzalutamide, whereas OS was not different between the two classes of agents among patients with AR-V7 negative CTCs. These clinical experiences suggest that AR-V7 assays may be useful predictor of abiraterone and enzalutamide resistance in men with metastatic CRPC with or without progression on prior enzalutamide or abiraterone. The prevalence of AR-V7 positivity is only 3% in patients prior to treatment with enzalutamide, abiraterone and taxanes, so the panel believes AR-V7 detection would not be useful to inform treatment decisions before these treatments are given. The panel recommends that use of AR-V7 tests can be considered to help guide selection of therapy in the post-abiraterone/enzalutimide metastatic CPC setting.
Rectal Cancer 2.2022	The clinical use of circulating tumor cells (CTC) or circulating tumor DNA (ctDNA) in rectal cancer is not included in this current NCCN guideline.
Small Bowel Adenocarcinoma 1.2022	The clinical use of circulating tumor cells (CTC) or circulating tumor DNA (ctDNA) in small bowel adenocarcinoma is not included in this current NCCN guideline.
Small Cell Lung Cancer 1.2023	The clinical use of circulating tumor cells (CTC) or circulating tumor DNA (ctDNA) in small cell lung cancer is not included in this current NCCN guideline.
Testicular Cancer 2.2022	The clinical use of circulating tumor cells (CTC) or circulating tumor DNA (ctDNA) in testicular cancer is not included in this current NCCN guideline.

Thyroid Carcinoma 2.2022	The clinical use of circulating tumor cells (CTC) or circulating tumor DNA (ctDNA) in thyroid carcinoma is not included in this current NCCN guideline.
Uterine Neoplasms 1.2022	The clinical use of circulating tumor cells (CTC) or circulating tumor DNA (ctDNA) in uterine neoplasm is not included in this current NCCN guideline.
Vulvar Cancer (Squamous Cell Carcinoma) Version 2.2022	The clinical use of circulating tumor cells (CTC) or circulating tumor DNA (ctDNA) in vulvar cancer is not included in this current NCCN guideline.

Regulatory Status
The CellSearch™ system (Janssen Diagnostics, formerly Veridex) is the only U.S. Food and Drug Administration (FDA) approved device for monitoring patients with metastatic disease and CTCs. In 2004, the CellSearch™ system was cleared by the FDA for marketing through the 510(k) process for monitoring metastatic breast cancer, In 2007 for monitoring metastatic colorectal cancer, and in 2008 for monitoring metastatic prostate cancer. The system uses automated instruments manufactured by Immunicon for sample preparation (Cell Tracks® AutoPrep) and analysis (CellSpotterAnalyzer®), together with supplies, reagents, and epithelial cell control kits manufactured by Veridex.

In August 2020, FoundationOne Liquid CDx (Foundation Medicine), a qualitative next generation sequencing-based diagnostic for circulating cell-free DNA in plasma,was approved by the FDA through the premarket approval process (P190032). The plasma test is approved as a companion diagnostic for selecting NSCLC patients who have EGFR exon 19 deletions and EGFR exon 21 L858R substitution variants, although information on multiple solid tumor biomarkers is also assessed. Genomic findings for biomarkers other than EGFR are not validated for choosing a particular corresponding treatment.. Prior versions of FoundationOne Liquid CDx were previously marketed as FoundationACT and FoundationOne laboratory developed test (LDT). Liquid biopsy for NSCLC is further addressed in medical policy 02.04.79 Circulating Tumor DNA for Management of Non-Small Cell Lung Cancer.

Tumor Type	**Biomarker(s) Detected**	**Therapy**
Non-small cell lung cancer (NSCLC)	*EGFR* Exon 19 deletions and *EGFR* Exon 21 L858R substitution	IRESSA® (gefitinib) TAGRISSO® (osimertinib) TARCEVA® (erlotinib)

In August 2020 Guardant360 CDx (Guardant Health) a qualitative next generation sequencing-based diagnostic of circulating cell-free DNA in plasma, was approved by the FDA through the premarket approval process (P200010). The plasma test is approved as a companion diagnostic for selecting NSCLC patients who have EGFR exon 19 deletions, L858R substitution variants, or T790M variants, for treatment with osimertinib (Tagrisso), although information on multiple solid tumor biomarkers is also assessed. Genomic findings for biomarkers other than EGFR are not validated for choosing a particular corresponding treatment. Prior version of Guardant360 CDx was previously marketed as Guardant360. Liquid biopsy for NSCLC is further addressed in medical policy 02.04.79 Circulating Tumor DNA for Management of Non-Small Cell Lung Cancer.

Clinical laboratories may develop and validate tests in-house and market them as a laboratory service; laboratory developed tests (LDTs) must meet the general regulatory standards of the Clinical Laboratory Improvement Amendments (CLIA). Circulating tumor DNA (ctDNA) and circulating tumor cells (CTCs) (liquid biopsy) for cancer management is available under the auspices of Clinical Laboratory Improvement Amendments. Laboratories that offer LDTs must be licensed by CLIA for high complexity testing. To date, the U.S. Food and Drug Administration (FDA) has chosen not to require any regulatory review of this test.

PRIOR APPROVAL

Not applicable.

POLICY

See Related Medical Policies

- 02.04.20 KRAS/NRAS and BRAF Mutational Analysis
- 02.04.55 Epidermal Growth Factor Receptor (EGFR) Mutation Analysis Excluding Non-Small Cell Lung Cancer
- 02.04.63 Expanded Genetic Panels to Identify Targeted Cancer Therapy
- 02.04.78 Molecular Analysis (Including Liquid Biopsy) for Targeted Therapy or Immunotherapy of Non-Small Cell Lung Cancer

Circulating Tumor Cell (CTC) Tests
Oncotype DX AR-V7 or AR-V7 Prostate Cancer testing from circulating tumor cells (CTCs) may be considered **medically necessary** for individuals with metastatic castrate resistant prostate cancer (mCRPC) considering second line therapy when **ALL** the following criteria are met:

- Progression* on androgen receptor – signaling inhibitor (ARSi) therapy enzalutamide (Xtandi) or abiraterone (Zytiag); **AND**

- AR-V7 will be assessed to guide subsequent therapeutic decision making.

*Progressive mCRPC is defined by the Prostate Cancer working Group 2 guidelines as the following: a minimum of 2 rising prostate-specific antigen (PSA) levels 1 or more weeks apart, new lesions by bone scintigraphy, and/or new or enlarging soft tissue lesions by computed tomography (CT) or magnetic resonance imaging (MRI).

The detection and use of circulating tumor cell (CTCs) enumeration in cancer management, not meeting the above criteria and for all other indications is considered **investigational**, including but not limited to the following commercially available tests, because the evidence is insufficient in determining the effects on net health outcomes:

- AR-V7 Prostate Cancer (except as indicated above)
- Cellmax First Sight CRC (Colorectal Cancer Early Detection Test)
- CellMax – PanCa Monitoring Test
- CellMax – Prostate Cancer Test
- CellSearch Circulating Tumor Cell Test (86152, 86153)
- Early Detection Test
- IVDiagnostics
- miR Sentinel Prostate Cancer Test (0343U)

Circulating Tumor DNA (ctDNA) Tests

The detection and use of liquid biopsy testing/circulating tumor DNA (ctDNA) for PIK3CA variant analysis (0177U, 81309) or FoundationOne Liquid CDx (0239U) to predict treatment response to Piqray (Alpelisib) may be considered **medically necessary** when the following criteria is met:

- The individual has recurrent or Stage IV hormone receptor – positive/HER2 negative breast cancer who have progressed on or after an endocrine- based regimen.

The detection and use of liquid biopsy testing/circulating tumor DNA (ctDNA) for PIK3CA variant analysis (0177U, 81309, 0239U) in the management of breast cancer is considered **investigational** when the above criteria are not met and for all other indications, because the evidence is insufficient in determining the effects on net health outcomes.

The detection and use of liquid biopsy testing/circulating tumor DNA (ctDNA) via comprehensive molecular profiling panel (FoundationOne Liquid CDx (0239U), Guardant360 CDx (0242U), or Guardant360 [0326U]) may be considered **medically necessary** when **ALL** of the following criteria are met:

- The individual has a diagnosis for one of the following:
  - Metastatic or advanced esophageal or esophagogastric junction cancer; **or**
  - Advanced gastric cancer; **or**
  - Locally advanced or metastatic pancreatic adenocarcinoma; **and**
- The individual is a candidate for anti-cancer therapy (chemotherapy or immunotherapy); **and**

- At least one of the following:
    - The individual is unable to undergo a tissue biopsy or an additional tissue biopsy due to documented medical reasons (i.e., invasive tissue sampling is contraindicated due to the individual's clinical condition); **or**
    - The individual does not have a biopsy-amendable lesion; **or**
    - There is insufficient tumor tissue available for molecular analysis.

The detection and use of liquid biopsy testing/circulating tumor DNA (ctDNA), including but not limited to the following, commercially available tests for all other cancer indications not indicated above is considered **investigational,** because the evidence is insufficient in determining the effects on net health outcomes.

> ***Note:*** *This policy does not address liquid biopsy testing/circulating tumor DNA for the management of non-small cell lung cancer, see medical policy 02.04.78 Molecular Analysis (Including Liquid Biopsy) for Targeted Therapy or Immunotherapy of Non-Small Cell Lung Cancer*

- Cancer Intercept
- CellMax – LBx Liquid Biopsy
- CellSearch HER2 Circulating Tumor Cell (CTC-HER2) test (0338U)
- CellSearch Cirulating Multiple Myeloma Cell (CMMC) Test (0337U)
- CellSearch Circulating Tumor Cell Test (86152, 86153)
- Circulogene
- ClearID Biomarker Expression Assays
- ClearID Breast Cancer
- ClearID Lung Cancer
- ClearID Solid Tumor Panel
- Colorectal Cancer Profile
- Colvera
- DAWN IO Melanoma
- FoundationOne Liquid CDx (except as indicated above)
- Galleri Test
- Guardant360 CDx (except as indicated above)
- Guardant360 (except as indicated above)
- Guardant Reveal (residual and recurrent disease in early- stage colorectal cancer)
- HelioLiver Test
- LiquidGx
- NavDX
- NeoLab Solid Tumor Liquid Biopsy
- OncoBEAM for Colorectal Cancer
- OncoBEAM for Melanoma
- Oncology (breast cancer), DNA, PIK3CA (except as indicated above)
- Oncotype DX AR-V7 Nucleus Detect (except as indicated above)
- PlasmaSelect64

- Signatera Bladder (0340U)
- Signatera Breast (0340U)
- Signatera Colon (0340U)
- Target Selector
- Tempus xF Liquid Biopsy Panel

PROCEDURE CODES AND BILLING GUIDELINES

To report provider services, use appropriate CPT* codes, Alpha Numeric (HCPCS level 2) codes, Revenue codes, and/or ICD diagnosis codes.

- 81455 Targeted genomic sequence analysis panel, solid organ or hematolymphoid neoplasm or disorder, 51 or greater genes (eg, ALK, BRAF, CDKN2A, CEBPA, DNMT3A, EGFR, ERBB2, EZH2, FLT3, IDH1, IDH2, JAK2, KIT, KRAS, MET, MLL, NOTCH1, NPM1, NRAS, PDGFRA, PDGFRB, PGR, PIK3CA, PTEN, RET), interrogation for sequence variants and copy number variants or rearrangements, or isoform expression or mRNA expression levels, if performed; DNA analysis or combined DNA and RNA analysis
- 81456 Targeted genomic sequence analysis panel, solid organ or hematolymphoid neoplasm or disorder, 51 or greater genes (eg, ALK, BRAF, CDKN2A, CEBPA, DNMT3A, EGFR, ERBB2, EZH2, FLT3, IDH1, IDH2, JAK2, KIT, KRAS, MET, MLL, NOTCH1, NPM1, NRAS, PDGFRA, PDGFRB, PGR, PIK3CA, PTEN, RET), interrogation for sequence variants and copy number variants or rearrangements, or isoform expression or mRNA expression levels, if performed; RNA analysis
- 81479 Unlisted molecular pathology procedure (May be utilized for AR-V7 Prostate Cancer, OncotypeDX AR-V7 Nucleus Detect, Guardant Reveal)
- 86152 Cell enumeration using immunologic selection and identification in fluid specimen (eg, circulating tumor cells in blood).
- 86153 Cell enumeration using immunologic selection and identification in fluid specimen (eg, circulating tumor cells in blood); physician interpretation and report.
- 81309 PIK3CA (phosphatidylinositol-4, 5-biphosphate 3-kinase, catalytic subunit alpha) (eg, colorectal and breast cancer) gene analysis, targeted sequence analysis (e.g., exons 7, 9, 20)
- 0091U Oncology (colorectal) screening, cell enumeration of circulating tumor cells, utilizing whole blood, algorithm, for the presence of adenoma or cancer, reported as a positive or negative result (CellMax – First Sight CRC [Colorectal Cancer Early Detection Test])
- 0177U Oncology (breast cancer), DNA, PIK3CA (phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha) gene analysis of 11 gene variants utilizing plasma, reported as PIK3CA gene mutation status

- 0239U Targeted genomic sequence analysis panel, solid organ neoplasm, cell-free DNA analysis of 311 or more genes, interrogation for sequence variants, including sequence variants, including substitutions, insertions, deletions, select rearrangements, and copy number variations (Includes FoundationOne Liquid CDx)
- 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 (Guardant360 CDx)
- 0326U Targeted genomic sequence analysis panel, solid organ neoplasm, cell-free circulating DNA analysis of 83 or more genes, interrogation for sequence variants, gene copy number amplifications, gene rearrangements, microsatellite instability and tumor mutational burden (Guardant360)
- 0333U Oncology (liver) surveillance for hepatocellular carcinoma (HCC) in high risk patients, analysis of methylation patterns on circulating cell-free DNA (cfDNA) plus measurement of serum AFP/AFP-L3 and oncoprotein des-gamma-carboxy-prothrombin (DCP), algorithm reported as normal or abnormal result (HelioLiver™ Test)
- 0337U Oncology (Plasma cell disorders and myeloma), circulating plasma cell immunologic selection, identification, morphologic characterization, and enumeration of plasma cells based on differential CD138, CD38, CD19, and CD45 protein biomarker expression peripheral blood (CellSearch Circulating Multiple Myeloma Cell (CMMC) Test)
- 0340U Oncology (pan-cancer), analysis of minimal residual disease (MRD) from plasma, with assays personalized to each patient based on prior next-generation sequencing of the patient's tumor and germline DNA, reported as absence or presence of MRD, with disease burden correlation, if appropriate. (Signatera)
- 0343U Oncology (prostate), exosome-based analysis of 442 small noncoding RNAs (sncRNAs) by quantitative reverse transcription polymerase chain reaction (RT-qPCR), urine, reported as molecular evidence of no-, low-, intermediate- or high-risk of prostate cancer (Urine Liquid Biopsy Test: miR Sentinel Prostate Cancer Test)
- 0365U Oncology (oropharyngeal), evaluation of 17 DNA biomarkers using droplet digital PCR (ddPCR), cell-free DNA, algorithm reported as a prognostic risk score for cancer recurrence (NavDx)
- 0357U Oncology (melanoma), artificial intelligence (AI)-enabled quantitative mass spectrometry analysis of 142 unique pairs of glycopeptide and product fragments, plasma, prognostic, and predictive algorithm reported as likely, unlikely, or uncertain benefit from immunotherapy agents (Dawn IO Melanoma)

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POLICY HISTORY		
Date	**Reason**	**Action**
September 2022	Annual Review	Policy Revised
November 2021	Interim Review	Policy Revised
September 2021	Annual Review	Policy Revised
September 2020	Annual Review	Policy Revised
September 2019	Annual Review	Policy Revised
December 2018	Interim Review	Policy Revised
September 2018	Annual Review	Policy Revised
September 2017	Annual Review	Policy Renewed
September 2016	Annual Review	Policy Renewed
October 2015	Annual Review	Policy Renewed
November 2014	Annual Review	Policy Renewed
January 2014	Annual Review	Policy Renewed
January 2013	Annual Review	Policy Renewed
January 2012	Annual Review	Policy Renewed
January 2011	Annual Review	Policy Renewed

New information or technology that would be relevant for Wellmark to consider when this policy is next reviewed may be submitted to:

Wellmark Blue Cross and Blue Shield
Medical Policy Analyst
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