2.04.153 Tumor-Informed Circulating Tumor DNA Testing for Cancer Management			
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Section:	2.0 Medicine	**Page:**	Page 1 of 36

Policy Statement

I. Tumor-informed circulating tumor DNA testing (e.g., Signatera) is considered **investigational** for all indications.

Note: For individuals enrolled in health plans subject to the Biomarker Testing Law (Health & Safety Code Section 1367.667 and the Insurance Code Section 10123.209), Centers for Medicare & Medicaid Services (CMS) Local Coverage Determination (LCD) may also apply. Please refer to the Medicare National and Local Coverage section of this policy and to MolDX: Minimal Residual Disease Testing for Cancer for reference.

NOTE: Refer to Appendix A to see the policy statement changes (if any) from the previous version.

Policy Guidelines

Coding

See the Codes table for details.

Description

This evidence review addresses the use of tumor-informed circulating tumor DNA (ctDNA) testing for cancer management. The purpose of tumor-informed ctDNA testing in individuals with cancer is to predict disease course to inform treatment decisions and to monitor for recurrence following treatment.

Summary of Evidence

For individuals with colorectal cancer (CRC) who receive tumor-informed circulating tumor DNA (ctDNA) testing with Signatera to guide treatment decisions and monitor for recurrence, the evidence includes a systematic review, 4 noncomparative studies (N = 1449), and 1 retrospective comparative study (N = 48). Relevant outcomes are overall survival, disease-specific survival, test validity, other test performance measures, change in disease status, morbid events, functional outcomes, health status measures, quality of life, and treatment-related mortality. The systematic review and nonrandomized studies have reported an association between ctDNA results measured at diagnosis, following surgery, during adjuvant therapy, and during surveillance after curative treatment and prognosis, but these studies are limited by a lack of comparison to tests used for the same purpose, imprecise estimates due to small sample sizes, and clinical heterogeneity of study populations. No study reported management changes made in response to ctDNA test results. A retrospective observational study found no advantage to surveillance with Signatera compared to standard surveillance conducted according to National Comprehensive Cancer Network (NCCN) guidelines (p>.99 for sensitivity and specificity compared to imaging). There is no direct evidence that the use of the test improves health outcomes, and indirect evidence is not sufficient to draw conclusions about clinical validity. The evidence is insufficient to determine that the technology results in an improvement in the net health outcome.

For individuals with breast cancer who receive tumor-informed ctDNA testing with Signatera to guide treatment decisions and monitor for recurrence, the evidence includes 2 noncomparative studies (N = 133). Relevant outcomes are overall survival, disease-specific survival, test validity, other test performance measures, change in disease status, morbid events, functional outcomes, health status measures, quality of life, and treatment-related mortality. One study evaluated Signatera testing for disease surveillance following primary treatment, and 1 reported the association of test results at different timepoints with response to neoadjuvant chemotherapy. Although the studies found an association of test results with prognosis, the studies are limited by a lack of comparison to tests used for the same purpose, imprecise estimates due to small sample sizes, and clinical heterogeneity of study populations. No study reported management changes made in response to ctDNA test results. There is no direct evidence that the use of the test improves health outcomes, and indirect evidence is not sufficient to draw conclusions about clinical validity. The evidence is insufficient to determine that the technology results in an improvement in the net health outcome.

For individuals with bladder cancer who receive tumor-informed ctDNA testing with Signatera to guide treatment decisions and monitor for recurrence, the evidence includes 1 uncontrolled prospective cohort study (N = 68), 1 retrospective cohort study (N = 102), and 1 retrospective subgroup analysis from a randomized controlled trial (N = 581). Relevant outcomes are overall survival, disease-specific survival, test validity, other test performance measure, change in disease status, morbid events, functional outcomes, health status measures, quality of life, and treatment-related mortality. The prospective study reported an association between Signatera test results at diagnosis, during chemotherapy treatment, and during surveillance following cystectomy to prognosis. The retrospective study reported an association between Signatera test results at diagnosis and during surveillance following cystectomy to prognosis; patients in this study did not receive chemotherapy. The retrospective subgroup analysis reported an association between test results and response to atezolizumab treatment. Study limitations, including a lack of comparison to tests used for the same purpose preclude drawing conclusions about clinical validity and usefulness. No study reported management changes made in response to ctDNA test results. There is no direct evidence that the use of the test improves health outcomes, and indirect evidence is not sufficient to draw conclusions about clinical validity. The evidence is insufficient to determine that the technology results in an improvement in the net health outcome.

For individuals with non-small cell lung cancer (NSCLC) who receive tumor-informed ctDNA testing with Signatera to guide treatment decisions and monitor for recurrence, the evidence includes 1 subgroup analysis of participants enrolled in a prospective observational study (N = 24). Relevant outcomes are overall survival, disease-specific survival, test validity, other test performance measures, change in disease status, morbid events, functional outcomes, health status measures, quality of life, and treatment-related mortality. Of 14 individuals with confirmed relapse, 13 (93%) had a positive ctDNA test (defined as at least 2 single-nucleotide variants detected). Of 10 individuals with no relapse after a median follow up of 775 days, (range 688 to 945 days), 1 had a positive ctDNA test (10%). This study's small sample size and lack of a comparator preclude drawing conclusions about clinical validity. There is no direct evidence that the use of the test improves health outcomes, and indirect evidence is not sufficient to draw conclusions about clinical validity. The evidence is insufficient to determine that the technology results in an improvement in the net health outcome.
For individuals with esophageal cancer who receive tumor-informed ctDNA testing with Signatera to guide treatment decisions and monitor for recurrence, the evidence includes 1 noncomparative, retrospective study (N = 17). Relevant outcomes are overall survival, disease-specific survival, test validity, other test performance measure, change in disease status, morbid events, functional outcomes, health status measures, quality of life, and treatment-related mortality. Patients who were ctDNA-positive before surgery had significantly poorer disease-free survival (DFS) ($p<.042$), with a median DFS of 32.0 months versus 63.0 months in ctDNA-negative preoperative patients. This study was limited by its small number sample size and retrospective design. There is no direct evidence that the use of the test improves health outcomes. Due to the study's limitations and lack of additional

supporting studies, the evidence is not sufficient to draw conclusions on clinical validity. Additionally, the management pathway for Signatera testing in esophageal cancer has not been clearly defined. The evidence is insufficient to determine that the technology results in an improvement in the net health outcome.

For individuals with solid tumors who receive tumor-informed ctDNA testing with Signatera to monitor response to immunotherapy, the evidence includes a subgroup analysis of individuals enrolled in a nonrandomized trial of pembrolizumab (N = 106). Relevant outcomes are overall survival, disease-specific survival, test validity, other test performance measures, change in disease status, morbid events, functional outcomes, health status measures, quality of life, and treatment-related mortality. The subgroup analysis evaluated Signatera testing to monitor response to immunotherapy in individuals with advanced solid tumors who were enrolled in a Phase II clinical trial of pembrolizumab. Lower-than-median ctDNA levels at baseline were associated with improved overall survival (adjusted hazard ratio [HR], 0.49; 95% confidence interval [CI], 0.29 to 0.83) and progression-free survival (adjusted HR, 0.54; 95% CI, 0.34 to 0.85). The study was limited by a small sample size, variability in results across different tumor types, and lack of a comparison to standard methods of monitoring response to treatment. There is no direct evidence that the use of the test improves health outcomes, and indirect evidence is not sufficient to draw conclusions about clinical validity. Additionally, the management pathway for Signatera testing for monitoring response to immunotherapy has not been clearly defined. The evidence is insufficient to determine that the technology results in an improvement in the net health outcome.

Additional Information
Not applicable.

Related Policies

- Circulating Tumor DNA and Circulating Tumor Cells for Cancer Management (Liquid Biopsy)
- Germline and Somatic Biomarker Testing (Including Liquid Biopsy) for Targeted Treatment in Breast Cancer (BRCA1, BRCA2, PIK3CA, Ki-67, RET, BRAF, ESR1, NTRK)
- Somatic Biomarker Testing (Including Liquid Biopsy) for Targeted Treatment in Non-Small-Cell Lung Cancer (EGFR, ALK, BRAF, ROS1, RET, MET, KRAS, NTRK) **(to be published)**

Benefit Application

Benefit determinations should be based in all cases on the applicable member health services contract language. To the extent there are conflicts between this Medical Policy and the member health services contract language, the contract language will control. Please refer to the member's contract benefits in effect at the time of service to determine coverage or non-coverage of these services as it applies to an individual member.

Some state or federal law may prohibit health plans from denying FDA-approved Healthcare Services as investigational or experimental. In these instances, Blue Shield of California may be obligated to determine if these FDA-approved Healthcare Services are Medically Necessary.

Regulatory Status

SB 535
Starting on July 1, 2022 (per CA law SB 535) for commercial plans regulated by the California Department of Managed Healthcare and California Department of Insurance (PPO and HMO), health care service plans and insurers shall not require prior authorization for biomarker testing, including biomarker testing for cancer progression and recurrence, if a member has stage 3 or 4

cancer. Health care service plans and insurers can still do a medical necessity review of a biomarker test and possibly deny coverage after biomarker testing has been completed and a claim is submitted (post service review).

SB 496
SB 496 requires health plans licensed under the Knox-Keene Act ("Plans"), Medi-Cal managed care plans ("MCPS"), and health insurers ("Insurers") to cover biomarker testing for the diagnosis, treatment, appropriate management, or ongoing monitoring of an enrollee's disease or condition to guide treatment decisions, as prescribed. The bill does not require coverage of biomarker testing for screening purposes. Restricted or denied use of biomarker testing for these purposes is subject to state and federal grievance and appeal processes. Where biomarker testing is deemed medically necessary, Plans and Insurers must ensure that the testing is provided in a way that limits disruptions in care.

Signatera Regulatory Information

Signatera is a laboratory developed test regulated under CLIA. Signatera has been developed and its performance characteristics determined by Natera, the CLIA-certified laboratory performing the test. The test has not been cleared or approved by the US Food and Drug Administration (FDA), but has received 3 Breakthrough Device Designations from FDA:

- In May 2019, Signatera was granted a Breakthrough Device Designation (BDD) for the detection of ctDNA in localized or advanced colorectal cancer patients to optimize the use of chemotherapy alone or in combination with durvalumab.
- A March 2021 press release announced that FDA granted 2 additional BDDs covering new intended uses.[1,]

Rationale

Background

The purpose of tumor-informed circulating tumor DNA (ctDNA) testing in individuals with cancer is to predict disease course to inform treatment decisions and to monitor for recurrence following treatment.

Signatera

Signatera is a tumor-specific ctDNA test. Tumor tissue obtained from either a diagnostic biopsy or surgically resected tissue is used to identify 16 single nucleotide variants found in the tumor but not in normal tissue and are likely to be present in all tumor cells regardless of tumor evolution. A custom assay of 16 tumor-specific clonal, somatic variants is generated for the individual and the resulting tumor signature can be monitored throughout the individual’s disease course. When the test is used for detection of recurrence following curative treatment, plasma samples with 2 or more out of these 16 variants detected above a predefined confidence threshold are deemed to be ctDNA-positive. When the test is used to monitor treatment response, evaluation is based on whether ctDNA levels increase or decrease from a baseline measurement. The test is intended to be used in conjunction with radiological assessment.

Literature Review

Evidence reviews assess whether a medical test is clinically useful. The first step in assessing a medical test is to formulate the clinical context and purpose of the test. A useful test provides information to make a clinical management decision that improves the net health outcome. That is, the balance of benefits and harms is better when the test is used to manage the condition than when another test or no test is used to manage the condition.

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 individuals 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 individuals managed with and without the test. Because these are intervention studies, the preferred evidence would be from randomized controlled trials (RCTs).

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.

Tumor-Informed Circulating Tumor DNA Testing with Signatera in Individuals with Colorectal Cancer

Clinical Context and Test Purpose

The purpose of Signatera testing in individuals who have colorectal cancer (CRC) is to inform treatment decisions and to monitor for recurrence following curative treatment.
The following PICO was used to select literature to inform this review.

Populations

The relevant populations of interest are individuals:

- With stage II or III CRC who have undergone surgical resection, or
- Who are being monitored for relapse following treatment for stage II or III CRC , or
- With metastatic (stage IV) CRC who have undergone surgical resection and are being evaluated for adjuvant chemotherapy and/or targeted therapy.

Interventions

The test being considered is circulating tumor DNA (ctDNA) testing with Signatera:

- Following surgery, to inform decisions about adjuvant chemotherapy or targeted therapy, or
- During disease surveillance after curative treatment, to identify metastatic relapse at an early timepoint, and aid in the selection of individuals who may benefit from early/adjuvant treatment.

Comparators

For individuals with stage II CRC , the current standard of care is not to routinely administer adjuvant chemotherapy. However, current National Comprehensive Cancer Network (NCCN) guidelines are that adjuvant chemotherapy can be considered in individuals with stage II CRC , using clinicopathologic characteristics to identify individuals who might benefit.

For individuals with stage III CRC , the current standard of care is to administer adjuvant chemotherapy routinely.

For individuals who are being monitored for relapse following treatment for stage II or III CRC , guidelines suggest monitoring carcinoembryonic antigen (CEA) every 3 to 6 months for 2 years, then every 6 months for a total of 5 years, as well as imaging every 6 to 12 months for 5 years.

For individuals with metastatic CRC who have undergone surgical resection, the current standard of care is routine individual checkups, periodic computed tomography scans, and monitoring of CEA level.

Outcomes

The general outcomes of interest are disease-specific survival, test accuracy and validity, and change in disease status. Specific outcomes of interest are recurrence risk, recurrence-free survival (RFS), and overall survival at follow-up.

Given that the majority of CRC recurrences occur within the first 3 years after surgical resection of the primary tumor and approximately 95% in the first 5 years, the timepoint of interest to assess recurrence is 3 to 5 years following surgical resection.

For individuals with stage II CRC who are being evaluated for adjuvant chemotherapy, given that the test will be used to *rule-in* stage II individuals for adjuvant chemotherapy, the performance characteristics of most interest are positive predictive value and specificity.

For individuals with stage III CRC who are being evaluated for adjuvant chemotherapy, given that the test will be used to *rule-out* individuals for adjuvant chemotherapy, the performance characteristics of most interest are negative predictive value and sensitivity. However, since the test would be used to select individuals who would not receive category 1 recommended treatment, direct evidence of improvement in outcomes is required. For individuals who are being monitored for relapse following treatment for CRC , recurrence at 3 to 5 years should be assessed.

Study Selection Criteria

For the evaluation of clinical validity of the Signatera test, studies that meet the following eligibility criteria were considered:

- Reported on the accuracy of the marketed version of the technology
- Included a suitable reference standard
- Individual/sample clinical characteristics were described
- Individual/sample selection criteria were described.

Systematic Reviews

Chidharla et al (2023) published a systematic review of 23 studies (N = 3568) investigating the use of ctDNA as a biomarker for minimal residual disease in patients with CRC after curative-intent surgery; only 3 of the included studies used the Signatera ctDNA assay and are described in more detail in the section below (Henriksen et al [2022]; Loupakis et al [2021]; Kotani et al [2023]).[2,] The results of this analysis demonstrated that ctDNA positivity after surgery was associated with a significantly higher risk of recurrence, with a pooled hazard ratio (HR) of 7.27 for all stages of CRC. Furthermore, post-adjuvant chemotherapy ctDNA positivity was associated with an even higher risk of recurrence (pooled HR, 10.59).

Nonrandomized Trials

Five nonrandomized studies, 4 of which were noncomparative, examined the association of Signatera testing to prognosis in individuals with CRC (Table 1). They differed in their study designs, populations (e.g., stage of disease), frequency and timing of standard care, outcome measures, and timing of follow up. Three studies evaluated the association between positive ctDNA results and prognosis in CRC (Table 2). These studies did not provide comparisons of ctDNA testing to standard methods of risk stratification for therapy selection, monitoring response to therapy, or early relapse detection. One retrospective study compared Signatera testing to other surveillance strategies in individuals with resected CRC.[3,] There are no RCTs, and no studies in which Signatera testing was used to guide treatment decisions.

Reinert et al (2019) enrolled 125 individuals with stage I to III CRC in a validation study of the Signatera assay.[4,] Plasma samples were collected before surgery, at 30 days following surgery, and every 3 months for up to 3 years. The recurrence rate at 3 years was 70% in individuals with a positive ctDNA test (7 of 10) compared to 11.9% (10 of 84) of those with a negative ctDNA test. In multivariate analyses, ctDNA status was associated with recurrence after adjusting for clinicopathological risk factors including stage, lymphovascular invasion, and microradical resection status.

Henriksen et al (2022) assessed the added benefit of serial ctDNA analysis; with samples taken at diagnosis, following surgery, during adjuvant therapy, and at follow up.[5,]

Loupakis et al (2021) evaluated the association of ctDNA with Signatera on survival outcomes in 112 individuals who had undergone resection for metastatic (stage IV) CRC.[6,] The study included an analysis of the sensitivity of Signatera testing to digital droplet polymerase chain reaction (PCR) testing but not to standard methods to identify recurrence, such as CEA and imaging.
Fakih et al (2022) directly compared Signatera testing to other surveillance strategies in individuals with resected CRC in a retrospective observational study (Table 3).[3,] This study was unique in that it used NCCN recommended guidelines for surveillance and ctDNA testing was performed at the same interval as standard surveillance with CEA and imaging. Test characteristics for Signatera were not significantly different from standard imaging techniques. Estimates were imprecise, with wide confidence intervals.

Kotani et al (2023) analyzed presurgical and postsurgical ctDNA levels in a large (N = 1039) prospective study that included patients with stage II to IV resectable CRC.[7,] After a median follow-up of 16.74 months, postsurgical ctDNA positivity at 4 weeks after surgery was associated with a significantly higher risk of recurrence (HR, 10.0; 95% CI, 7.7 to 14; p<.0001), and identified patients with high-risk stage II or III CRC who derived a benefit from adjuvant chemotherapy (HR, 6.59; 95% CI, 3.53 to 12.3; p<.0001). For both outcomes, trends were observed across all pathological stages evaluated.

Study limitations are shown in Tables 4 and 5. Major limitations include a lack of comparison to tests used for the same purpose, imprecise estimates due to small sample sizes, and clinical heterogeneity of study populations.

Table 1. Nonrandomized Studies of Signatera Testing in Colorectal Cancer - Study Characteristics

Study	Test Purpose	Study Population	Setting	Reference Standard	Threshold for Positive Index Test	Timing of Reference and Index Tests	Blinding of Assessors
Reinert et al (2019)[4,]	1. Risk stratification 2. Monitoring response to adjuvant chemotherapy 3. Early relapse detection	130 individuals with stages I to III CRC; treated from May 1, 2014 to January 31, 2017	Multicenter, Denmark	CEA and CT imaging	2 or more variants detected out of 16	Before and after surgery, during and after adjuvant chemotherapy, and during surveillance Sample at Day 30 following surgery; individuals were followed up for a median of 12.5 months	Yes
Henriksen et al (2022)[5,]	1. Risk stratification 2. Monitoring response to adjuvant chemotherapy 3. Early relapse detection Assessed added benefit of serial measurements	168 individuals with stage III CRC treated with curative intent between 2014 and 2019	Multicenter, Spain and Denmark	CEA analysis-thresholds set according to national guidelines and CT imaging	ctDNA detected-greater or equal to 2 variants detected out of 16	Median sampling 2 weeks after surgery (IQR, 2 to 4 weeks); postoperative plasma samples (within 2-4 weeks) prior. Plasma samples were also collected during and after adjuvant	Yes

Study	Test Purpose	Study Population	Setting	Reference Standard	Threshold for Positive Index Test	Timing of Reference and Index Tests	Blinding of Assessors
						therapy; individuals were followed up for a median of 35 months.	
Loupakis et al (2021)[6],	1. Risk stratification following surgery	112 individuals with stage IV CRC who had undergone resection with curative intent as part of the PREDATOR clinical trial	Italy	Radiological imaging	ctDNA detected-greater or equal to 2 variants detected out of 16	Plasma samples collected at the first time point and at the time of radiologic evidence of progressive disease or at the last follow-up; individuals were followed for a median of 10.7 months	Yes
Fakih et al (2022)[3],	1. Risk stratification following surgery	48 individuals with stage II to IV CRC who underwent surveillance with Signatera and underwent curative resections between 2019 and 2021	US, single center, retrospective	Confirmed recurrence, defined as a positive ctDNA finding or a finding on imaging confirmed by biopsy, CEA level elevation, or subsequent tumor radiographic dynamics	Any positive assay finding more than 4 weeks after definitive surgery	Standard surveillance strategy included ctDNA every 3 months for 2 years and then every 6 months for 3 years. CEA at the same interval as the ctDNA assay. Imaging studies performed within NCCN guidelines and included yearly CT scans for 5 years for low-risk stage II disease and every 6 months for 2 years and then every year for 3 years for high-risk stage II and III disease. Imaging studies were performed every 3 months for 2 years and then every 6 months for 3 years for	No

Study	Test Purpose	Study Population	Setting	Reference Standard	Threshold for Positive Index Test	Timing of Reference and Index Tests	Blinding of Assessors
						resected stage IV disease.	
Kotani et al (2023) [7,]	1. Risk stratification 2. Monitoring response to adjuvant chemotherapy 3. Early relapse detection	1,039 individuals with stage II to IV or recurrent CRC who underwent surveillance with Signatera and underwent curative resections through June 2022	Japan	NR	Any positive assay finding more than 4 weeks after definitive surgery	Samples collected 4 weeks after surgery, as well as 12 weeks after surgery for some patients	No

CEA: carcinoembryonic antigen; CRC: colorectal cancer; CT: computerized tomography; ctDNA:circulating tumor DNA ; IQR: interquartile range; NCCN: National Comprehensive Cancer Network; NR: not reported.

Table 2. Recurrence Rates by Risk Category in Nonrandomized Studies of Signatera in Colorectal Cancer

Study	Mean Recurrence Rate (95% CI)	
	ctDNA Positive	**ctDNA Negative**
Reinert et al (2019)[4,]	7/10; 70% (34.2% to 93.1%)	10/84; 11.9% (6.3% to 20.1%)
HR for recurrence following surgery (95% CI)	7.2 (2.7 to 19.0); p<.001	
HR for recurrence following adjuvant chemotherapy (95% CI)	17.5 (5.4 to 56.5); p<.001	
Henriksen et al (2022)[5,]	16/20 (80%)	22/120 (18%)
HR for RFS (95% CI)	7.0 (3.7 to 13.5); p<.001	
Loupakis et al (2021)[6,]	59/61 (96.7%)	NR/51 Number with recurrences not reported; 49 of 51 were alive at data cutoff
HR for RFS (95% CI)	5.8 (3.5 to 9.7); p<.001	
HR for OS (95% CI)	16.0 (3.9 to 68.0); p<.001	
Kotani et al (2023) [7,]	187/1039 (18.0%)	852/1,039 (82%)
HR for DFS (95% CI)	10 (7.7 to 14); p<.001	
HR for benefit with adjuvant chemotherapy (95% CI)	6.59 (3.53 to 12.3); p<.0001	

CI: confidence interval; ctDNA: circulating tumor DNA; DFS:disease-free survival; HR: hazard ratio; NR: not reported; OS: overall survival; RFS: recurrence-free survival.

Table 3. Retrospective Comparison of Signatera to Other Surveillance Strategies in Resected Colorectal Cancer

Study	Sensitivity	Specificity	PPV	NPV	Median Time to Recurrence, months
Fakih et al (2022)[3,]					
Signatera Testing	53.3 (27.4 to 77.7)	100 (87.0 to 100)	100 (59.8 to 100)	82.5 (66.6 to 92.1)	14.3

Study	Sensitivity	Specificity	PPV	NPV	Median Time to Recurrence, months
Imaging	60.0 (32.9 to 82.5)	96.9 (82.5 to 99.8)	90.0 (54.1 to 99.5)	84.2 (68.1 to 93.4)	15.0
CEA	20.0 (5.3 to 48.6)	90.9 (74.5 to 97.6)	50.0 (13.9 to 86.1)	71.4 (55.2 to 83.8)	NA
CEA plus imaging	73.3 (44.8 to 91.1)	87.9 (70.9 to 96.0)	73.3 (44.8 to 91.1)	87.9 (70.9 to 96.0)	15.0
P-value	>.99	>.99	NA	NA	.45
Signatera vs. imaging	.55	.13			.79
Signatera vs. imaging plus CEA	.13	.25			NA
Signatera vs. CEA					

CEA: carcinoembryonic antigen; NA: not assessed; NPV: negative predictive value; PPV: positive predictive value.

Table 4. Study Relevance Limitations

Study	Population[a]	Intervention[b]	Comparator[c]	Outcomes[d]	Duration of Follow-Up[e]
Reinert et al (2019)[4],	1. Included individuals with stage I through III colorectal cancer		3. No comparator	1. Overall survival not assessed	1. Follow up for recurrence was under 3 years (median 12.5 months)
Henriksen et al (2022)[5],			3. No comparator		1. Follow up for recurrence was under 3 years (median 35 months)
Loupakis et al (2021)[6],			3. No comparator		1. Follow up for recurrence was under 3 years (median 10.7 months)
Fakih et al (2022)[3],				1. Survival outcomes not assessed	
Kotani et al (2023) [7],			3. No comparator	1. Overall survival not assessed	1. Follow up for recurrence was under 3 years (median 16.74 months)

The study limitations stated in this table are those notable in the current review; this is not a comprehensive gaps assessment.
[a] Population key: 1. Intended use population unclear; 2. Clinical context is unclear; 3. Study population is unclear; 4. Study population not representative of intended use.
[b] Intervention key: 1. Classification thresholds not defined; 2. Version used unclear; 3. Not intervention of interest.
[c] Comparator key: 1. Classification thresholds not defined; 2. Not compared to credible reference standard; 3. Not compared to other tests in use for same purpose.
[d] Outcomes key: 1. Study does not directly assess a key health outcome; 2. Evidence chain or decision model not explicated; 3. Key clinical validity outcomes not reported (sensitivity, specificity and predictive values); 4. Reclassification of diagnostic or risk categories not reported; 5. Adverse events of the test not described (excluding minor discomforts and inconvenience of venipuncture or noninvasive tests).
[e] Follow-Up key: 1. Follow-up duration not sufficient with respect to natural history of disease (true positives, true negatives, false positives, false negatives cannot be determined).

Table 5. Study Design and Conduct Limitations

Study	Selection[a]	Blinding[b]	Delivery of Test[c]	Selective Reporting[d]	Data Completeness[e]	Statistical[f]
Reinert et al (2019)[4,]	1. individual selection not described					Multiple subgroup analyses, small numbers of individuals with positive ctDNA tests.
Henriksen et al (2022)[5,]			2. Standard-of-care imaging frequency differed between the Spanish (every 6 months) and Danish (at month 12 and 36) cohort.			Small numbers of individuals with positive ctDNA tests.
Loupakis et al (2021)[6,]						Small numbers of individuals with positive ctDNA tests.
Fakih et al (2022)[3,]						
Kotani et al (2023) [7,]	1. individual selection not described					

ctDNA: circulating tumor DNA.
The study limitations stated in this table are those notable in the current review; this is not a comprehensive gaps assessment.
[a] Selection key: 1. Selection not described; 2. Selection not random or consecutive (i.e., convenience).
[b] Blinding key: 1. Not blinded to results of reference or other comparator tests.
[c] Test Delivery key: 1. Timing of delivery of index or reference test not described; 2. Timing of index and comparator tests not same; 3. Procedure for interpreting tests not described; 4. Expertise of evaluators not described.
[d] Selective Reporting key: 1. Not registered; 2. Evidence of selective reporting; 3. Evidence of selective publication.
[e] Data Completeness key: 1. Inadequate description of indeterminate and missing samples; 2. High number of samples excluded; 3. High loss to follow-up or missing data.
[f] Statistical key: 1. Confidence intervals and/or p values not reported; 2. Comparison to other tests not reported.

Section Summary: Tumor-Informed Circulating Tumor DNA Testing with Signatera in Individuals with Colorectal Cancer

For individuals with CRC who receive tumor-informed ctDNA testing with Signatera to guide treatment decisions and monitor for recurrence, the evidence includes a systematic review and 4 noncomparative studies (N = 1,449) and 1 retrospective comparative study (N = 48). The systematic review and nonrandomized studies have reported an association between ctDNA results measured at diagnosis, following surgery, during adjuvant therapy, and during surveillance after curative treatment and prognosis, but these studies are limited by a lack of comparison to tests used for the same purpose, imprecise estimates due to small sample sizes, and clinical heterogeneity of study populations. No study reported management changes made in response to ctDNA test results. A retrospective observational study found no advantage to surveillance with Signatera compared to standard surveillance conducted according to NCCN guidelines (p>.99 for sensitivity and specificity compared to imaging). There is no direct evidence that the use of the test improves health outcomes, and indirect evidence is not sufficient to draw conclusions about clinical validity.

Tumor-Informed Circulating Tumor DNA Testing with Signatera in Individuals with Breast Cancer

Clinical Context and Test Purpose

The purpose of Signatera testing in individuals with breast cancer is to predict disease course (e.g., aggressiveness, risk of recurrence, death) and inform treatment decisions, and to monitor for recurrence following curative treatment.

The following PICO was used to select literature to inform this review.

Populations

The population of interest is individuals with breast cancer, or those who have been treated for breast cancer and are being monitored for recurrence.

Interventions

The test being considered is ctDNA testing with Signatera:

- At diagnosis to inform decisions about neoadjuvant chemotherapy, or
- After surgery to inform decisions about adjuvant treatment, or
- Following curative treatment, to monitor for recurrence.

Comparators

- Decisions about neoadjuvant and adjuvant chemotherapy are based on clinicopathological risk factors.
- Guidelines for disease surveillance following breast cancer treatment recommend regular imaging and physical examinations, and additional testing upon presentation of symptoms.

Outcomes

The general outcomes of interest are disease-specific survival, test accuracy and validity, and change in disease status. Specific outcomes of interest are recurrence risk, RFS, and overall survival at follow-up.

The specific outcomes of interest depend on the proposed purpose of testing in individuals with breast cancer.

- If used for risk stratification to *rule-out* individuals for neoadjuvant chemotherapy at diagnosis or adjuvant treatment following surgery, the performance characteristics of most interest are negative predictive value and sensitivity.
- If used for risk stratification to *rule-in* individuals for neoadjuvant chemotherapy at diagnosis or adjuvant treatment following surgery, the performance characteristics of most interest are positive predictive value and specificity.

If used for disease surveillance following primary treatment, beneficial outcomes of a true positive test would be earlier detection of metastasis and initiation of treatment. Harmful outcomes of a false positive test would be undergoing unnecessary or incorrect treatment, and experiencing adverse effects of such treatment.

See also Evidence review 2.04.36 for additional discussion of outcomes in breast cancer risk assessment studies.

Study Selection Criteria

For the evaluation of clinical validity of the Signatera test, studies that meet the following eligibility criteria were considered:

- Reported on the accuracy of the marketed version of the technology
- Included a suitable reference standard
- Individual/sample clinical characteristics were described
- Individual/sample selection criteria were described.

Nonrandomized Trials

Two noncomparative studies reported the association of Signatera testing with survival outcomes in breast cancer (Table 6). There are no RCTs, and no studies in which Signatera testing was used to guide treatment decisions.

Coombes et al (2019) evaluated Signatera for disease surveillance in 49 individuals who had received surgery and adjuvant therapy for stage I to III breast cancer of various subtypes.[8,] Signatera detected ctDNA in 16 of 18 individuals who subsequently relapsed, and the presence of ctDNA test was associated with poorer prognosis (Table 7).

Magbanua et al (2021) evaluated ctDNA clearance as a predictor of response to neoadjuvant chemotherapy (NAC) in 84 individuals with nonmetastatic breast cancer who were enrolled in the I-SPY2 trial.[9,] In the population as a whole, ctDNA positivity decreased during the course of NAC, from 73% before treatment (T0), to 35% at 3 weeks (T1), to 14% at the inter-regimen time point (T2), and down to 9% after NAC (T3). Hazard ratios for recurrence at each of these timepoints are shown in Table 7 and indicate that positive predictive value increased over time.

Study limitations are shown in Tables 8 and 9. Major limitations of both studies include a lack of comparison to standard methods of monitoring, and heterogeneity in the study populations.

Table 6. Nonrandomized Studies of Signatera Testing in Breast Cancer - Study Characteristics

Study	Test Purpose	Study Population	Study Design and Setting	Reference Standard	Threshold for Positive Index Test	Timing of Reference and Index Tests	Blinding of Assessors
Coombes et al (2019)[8,]	Relapse detection following primary treatment	49 individuals with stage I to III breast cancer who had undergone surgery and adjuvant chemotherapy; 34 HR–positive/HER2-negative, 8 HER2-positive, 7 TNBC	Prospective cohort, multicenter, UK	Cancer antigen 15-3 serum testing, CT imaging	2 or more variants detected out of 16	Plasma samples every 6 months for up to 4 years	Yes
Magbanua et al (2021)[9,]	Response to neoadjuvant chemotherapy	84 individuals with ≥ 2.5 cm nonmetastatic stage II/III breast cancer	Retrospective analysis of samples prospectively collected as part of the I-SPY2 TRIAL	Radiological imaging	2 or more variants detected out of 16	Plasma samples collected before, during, and after neoadjuvant chemotherapy	Yes

CT: computerized tomography; HR: hormone receptor; HER2: human epidermal growth factor receptor 2; TNBC: triple-negative breast cancer.

Table 7. Nonrandomized Studies of Signatera Testing in Breast Cancer - Study Results

Study	Initial N	Final N	Excluded Samples	Recurrence Rate	Median Time to Recurrence, months (range)	Clinical Validity			
						Sensitivity	*Specificity*	*PPV*	*NPV*
Coombes et al (2019)[8],	197	49	148	18/49 (36.7%)	8.9 (0.5 to 24.0)	16/18 (89%)	31/31 (100%)	NR	NR
HR (95% CI) for RFS (first postsurgical sample)	11.8 (4.3 to 32.5), p<.001								
HR (95% CI) for RFS (any follow up sample)	35.8 (7.9 to 161.3), p<.001								
Magbanua et al (2021)[9],	84	75	9	NA	NA	NR	NR	4/6 (67%)	50/54 (93%)
HR (95% CI) for recurrence (T0, baseline)	4.11 (0.52 to 32.4)								
HR (95% CI) for RFS (T1, 3 weeks after therapy initiation)	4.5 (1.2 to 17.4)								
HR (95% CI) for RFS (T2, between regimens)	5.4 (1.3 to 22.5)								
HR (95% CI) for RFS (T3, after neoadjuvant chemotherapy)	11.5 (2.9 to 46.1)								

CI: confidence interval; HR: hazard ratio; NA: not applicable; NPV: negative predictive value; NR: not reported; PPV: positive predictive value; RFS: recurrence-free survival.

Table 8. Study Relevance Limitations

Study	Population[a]	Intervention[b]	Comparator[c]	Outcomes[d]	Duration of Follow-Up[e]
Coombes et al (2019)[8],	2. Study population included a mix of individuals with stage I to III breast cancer		3. Not compared to tests used for the same purpose		
Magbanua et al (2021)[9],			3. Not compared to tests used for the same purpose		

The study limitations stated in this table are those notable in the current review; this is not a comprehensive gaps assessment.
[a] Population key: 1. Intended use population unclear; 2. Clinical context is unclear; 3. Study population is unclear; 4. Study population not representative of intended use.
[b] Intervention key: 1. Classification thresholds not defined; 2. Version used unclear; 3. Not intervention of interest.
[c] Comparator key: 1. Classification thresholds not defined; 2. Not compared to credible reference standard; 3. Not compared to other tests in use for same purpose.
[d] Outcomes key: 1. Study does not directly assess a key health outcome; 2. Evidence chain or decision model not explicated; 3. Key clinical validity outcomes not reported (sensitivity, specificity and predictive values); 4. Reclassification of diagnostic or risk categories not reported; 5. Adverse events of the test not described (excluding minor discomforts and inconvenience of venipuncture or noninvasive tests).
[e] Follow-Up key: 1. Follow-up duration not sufficient with respect to natural history of disease (true positives, true negatives, false positives, false negatives cannot be determined).

Table 9. Study Design and Conduct Limitations

Study	Selection[a]	Blinding[b]	Delivery of Test[c]	Selective Reporting[d]	Data Completeness[e]	Statistical[f]
Coombes et al (2019)[8,]					1. Confidence intervals for test characteristics not reported; small number of positive ctDNA tests	
Magbanua et al (2021)[9,]	2. Retrospective analysis				1. Confidence intervals for test characteristics not reported; small number of positive ctDNA tests	

ctDNA: circulating tumor DNA.
The study limitations stated in this table are those notable in the current review; this is not a comprehensive gaps assessment.
[a] Selection key: 1. Selection not described; 2. Selection not random or consecutive (i.e., convenience).
[b] Blinding key: 1. Not blinded to results of reference or other comparator tests.
[c] Test Delivery key: 1. Timing of delivery of index or reference test not described; 2. Timing of index and comparator tests not same; 3. Procedure for interpreting tests not described; 4. Expertise of evaluators not described.
[d] Selective Reporting key: 1. Not registered; 2. Evidence of selective reporting; 3. Evidence of selective publication.
[e] Data Completeness key: 1. Inadequate description of indeterminate and missing samples; 2. High number of samples excluded; 3. High loss to follow-up or missing data.
[f] Statistical key: 1. Confidence intervals and/or p values not reported; 2. Comparison to other tests not reported.

Section Summary: Tumor-Informed Circulating Tumor DNA Testing with Signatera in Individuals with Breast Cancer

For individuals with breast cancer who receive tumor-informed ctDNA testing with Signatera to guide treatment decisions and monitor for recurrence, the evidence includes 2 noncomparative studies (N = 133). One study evaluated Signatera testing for disease surveillance following primary treatment, and 1 reported the association of test results at different timepoints with response to neoadjuvant chemotherapy. Although the studies found an association of test results with prognosis, the studies are limited by a lack of comparison to tests used for the same purpose, imprecise estimates due to small sample sizes, and clinical heterogeneity of study populations. No study reported management changes made in response to ctDNA test results. There is no direct evidence that the use of the test improves health outcomes, and indirect evidence is not sufficient to draw conclusions about clinical validity.

Tumor-Informed Circulating Tumor DNA Testing with Signatera in Individuals with Bladder Cancer

Clinical Context and Test Purpose

The purpose of Signatera testing in individuals with bladder cancer is to predict disease course to inform treatment decisions and to monitor for recurrence following curative treatment.
The following PICO was used to select literature to inform this review.

Populations

The relevant population of interest is individuals with bladder cancer, or those who have been treated for bladder cancer and are being monitored for recurrence.

Interventions

The test being considered is ctDNA testing with Signatera:

- At diagnosis, to identify individuals at low risk of recurrence after cystectomy who may be eligible for cystectomy without neoadjuvant chemotherapy, or

- After chemotherapy before cystectomy, to determine treatment response and inform treatment decisions (e.g., additional cycles of chemotherapy or other therapeutic strategies), or
- During disease surveillance after cystectomy, to identify metastatic relapse after cystectomy at an early time point, and aid in the selection of individuals who may benefit from early/adjuvant treatment. For individuals with bladder cancer who are being evaluated for adjuvant chemotherapy, given that the test will be used to *rule-in* individuals for adjuvant chemotherapy, the performance characteristics of most interest are positive predictive value and specificity.

Comparators

- Urine testing, cystoscopy, and radiographic imaging are used for disease monitoring in individuals with bladder cancer.
- Detection of relapse and monitoring of response to treatment in the metastatic setting is performed by standard computed tomography scan.

Outcomes

The general outcomes of interest are disease-specific survival, test accuracy and validity, and change in disease status. Specific outcomes of interest are recurrence risk, RFS, and overall survival at follow-up.

If used to *rule in* individuals with bladder cancer who would be likely to benefit from adjuvant chemotherapy, the performance characteristics of most interest are positive predictive value and specificity.

If used to *rule out* patients with bladder cancer who could forego adjuvant chemotherapy, the performance characteristics of most interest are negative predictive value and sensitivity. However, since the test would be used to select individuals who would not receive category 1 recommended treatment, direct evidence of improvement in outcomes is required.

Study Selection Criteria

For the evaluation of clinical validity of the Signatera test, studies that meet the following eligibility criteria were considered:

- Reported on the accuracy of the marketed version of the technology
- Included a suitable reference standard
- Individual/sample clinical characteristics were described
- Individual/sample selection criteria were described.

Nonrandomized Trials

Two nonrandomized studies have reported an association between Signatera testing and prognosis in bladder cancer (Tables 10 and 11).

Christensen et al (2019) assessed the association of ctDNA with prognosis in 68 individuals with localized advanced bladder cancer who were receiving neoadjuvant chemotherapy before cystectomy (median follow-up of 21 months).[10,] Data from a 68-month follow-up of this cohort were reported by Lindskrog et al (2023).[11,] Additionally, Lingskrog et al (2023) reported on the association of ctDNA with prognosis in a separate cohort of 102 patients who did not receive neoadjuvant chemotherapy and had ctDNA testing before and after cystectomy (median follow-up of 72 months). Results demonstrated that ctDNA was prognostic regardless of whether or not patients received neoadjuvant chemotherapy before cystectomy.

Powles et al (2021) reported the association of a positive Signatera test to treatment response in 581 individuals who had undergone surgery for urothelial cancer and were enrolled in a RCT of

atezolizumab versus observation.[12,] Study participants who were positive for ctDNA had improved disease-free survival (DFS) and overall survival in the atezolizumab arm versus the observation arm (DFS HR, 0.58 [95% CI, 0.43 to 0.79]; p=.0024 and overall survival HR, 0.59 [95% CI, 0.41 to 0.86]). No difference in DFS or overall survival between treatment arms was noted for patients who were negative for ctDNA. At 2-year follow up, ctDNA status remained prognostic and no relapses were observed in the ctDNA-negative patients at baseline and after neoadjuvant therapy.[13,]

The major limitation of these studies was lack of comparison to other tests used for the same purpose (Tables 12 and 13).

Table 10. Nonrandomized Studies of Signatera Testing in Bladder Cancer - Study Characteristics

Study	Study Population	Study Design and Setting	Reference Standard	Threshold for Positive Index Test	Timing of Reference and Index Tests	Blinding of Assessors
Lindskrog et al (2023)[11,]	102 individuals with muscle-invasive bladder cancer who underwent cystectomy between 2001 and 2014 and did not receive neoadjuvant chemotherapy	Retrospective, one University Hospital, Denmark	Radiological imaging	Greater or equal to 2 variants detected out of 16	Surveillance according to European Guidelines. Blood samples collected before and after cystectomy. Median follow-up of 72 months after cystectomy.	NR
Christensen et al (2019) [10,11,]	68 individuals with muscle-invasive bladder cancer who were receiving neoadjuvant chemotherapy before cystectomy between 2013 and 2017	Prospective, one University Hospital, Denmark	Radiological imaging	Greater or equal to 2 variants detected out of 16	Surveillance according to European Guidelines. Blood samples collected at uniformly scheduled clinical visits and before each chemotherapy cycle. Median follow-up of 21 months after cystectomy (published by Christensen et al [2019]). Median follow-up of 68 months after cystectomy (published by Lindskrog et al [2023]).	Yes
Powles et al (2021)[12,]	581 individuals with urothelial cancer from a randomized Phase III trial of	Retrospective	Radiological imaging	Greater or equal to 2 variants detected out of 16	Post-surgical plasma samples were collected and tested at baseline and 6	No

Study	Study Population	Study Design and Setting	Reference Standard	Threshold for Positive Index Test	Timing of Reference and Index Tests	Blinding of Assessors
	adjuvant atezolizumab vs. observation who had undergone surgery and were evaluable for ctDNA				weeks after randomization and individuals were followed up for a median of 23 months	

ctDNA: circulating tumor DNA; NR: not reported.

Table 11. Recurrence Rates by Risk Category in Nonrandomized Studies of Signatera in Bladder Cancer

Study	Mean Recurrence Rate (95% CI)	
	ctDNA Positive	ctDNA Negative
Lindskrog et al (2023) [11,]		
At diagnosis before cystectomy	44/96 (46%)	52/96 (54%)
Adjusted HR (95% CI) for recurrence at 72-month follow-up	3.4 (1.7 to 6.8); p=.0005	
After cystectomy	15/34 (44%)	19/34 (56%)
Adjusted HR (95% CI) for recurrence at 72-month follow-up	17.8 (3.9 to 81.2); p=.0002	
Christensen et al (2019) [10,11,]		
At diagnosis before chemotherapy	11/24 (46%)	1/35 (3%)
Adjusted HR for recurrence at 21-month follow-up	29.1; p=.001	
Adjusted HR (95% CI) for recurrence at 68-month follow-up	15.6 (3.5 to 69); p=.0003	
After chemotherapy before cystectomy	6/8 (75%)	6/55 (11%)
Adjusted HR for recurrence at 21-month follow-up	12.0; p<.001	
Adjusted HR (95% CI) for recurrence at 68-month follow-up	15.2 (5 to 46.8); p<.0001	
During disease surveillance after cystectomy	13/17 (76%)	0/47 (0%)
Adjusted HR for recurrence at 21-month follow-up	129.6; p<.001	
Adjusted HR (95% CI) for recurrence at 68-month follow-up	37.7 (8.5 to 167.1); p<.0001	
Powles et al (2021)[12,]		
Following surgery (cycle 1 day 1)		
HR (95% CI) for DFS	6.3 (4.45 to 8.92); p<.0001	
6 weeks after randomization (cycle 3 day 1)		
HR (95% CI) for DFS	8.65 (5.67 to 13.18); p<.0001	

CI: confidence interval; ctDNA: circulating tumor DNA; DFS: disease-free survival; HR: hazard ratio; NR: not reported.

Table 12. Study Relevance Limitations

Study	Population[a]	Intervention[b]	Comparator[c]	Outcomes[d]	Duration of Follow-Up[e]
Lindskrog et al (2023)[11,]			3. Not compared to tests used for the same purpose		
Christensen et al (2019) [10,]			3. Not compared to tests used for the same purpose		

Study	Population[a]	Intervention[b]	Comparator[c]	Outcomes[d]	Duration of Follow-Up[e]
Powles et al (2021)[12,]	.		3. Not compared to tests used for the same purpose		

The study limitations stated in this table are those notable in the current review; this is not a comprehensive gaps assessment.
[a] Population key: 1. Intended use population unclear; 2. Clinical context is unclear; 3. Study population is unclear; 4. Study population not representative of intended use.
[b] Intervention key: 1. Classification thresholds not defined; 2. Version used unclear; 3. Not intervention of interest.
[c] Comparator key: 1. Classification thresholds not defined; 2. Not compared to credible reference standard; 3. Not compared to other tests in use for same purpose.
[d] Outcomes key: 1. Study does not directly assess a key health outcome; 2. Evidence chain or decision model not explicated; 3. Key clinical validity outcomes not reported (sensitivity, specificity and predictive values); 4. Reclassification of diagnostic or risk categories not reported; 5. Adverse events of the test not described (excluding minor discomforts and inconvenience of venipuncture or noninvasive tests).
[e] Follow-Up key: 1. Follow-up duration not sufficient with respect to natural history of disease (true positives, true negatives, false positives, false negatives cannot be determined).

Table 13. Study Design and Conduct Limitations

Study	Selection[a]	Blinding[b]	Delivery of Test[c]	Selective Reporting[d]	Data Completeness[e]	Statistical[f]
Lindskrog et al (2023)[11,]		Blinding not described.				
Christensen et al (2019) [10,]						1. Confidence intervals for hazard ratios not reported.
Powles et al (2021)[12,]						

The study limitations stated in this table are those notable in the current review; this is not a comprehensive gaps assessment.
[a] Selection key: 1. Selection not described; 2. Selection not random or consecutive (i.e., convenience).
[b] Blinding key: 1. Not blinded to results of reference or other comparator tests.
[c] Test Delivery key: 1. Timing of delivery of index or reference test not described; 2. Timing of index and comparator tests not same; 3. Procedure for interpreting tests not described; 4. Expertise of evaluators not described.
[d] Selective Reporting key: 1. Not registered; 2. Evidence of selective reporting; 3. Evidence of selective publication.
[e] Data Completeness key: 1. Inadequate description of indeterminate and missing samples; 2. High number of samples excluded; 3. High loss to follow-up or missing data.
[f] Statistical key: 1. Confidence intervals and/or p values not reported; 2. Comparison to other tests not reported.

Section Summary: Tumor-Informed Circulating Tumor DNA Testing with Signatera in Individuals with Bladder Cancer

For individuals with bladder cancer who receive tumor-informed ctDNA testing with Signatera to guide treatment decisions and monitor for recurrence, the evidence includes 1 uncontrolled prospective cohort study (N = 68), 1 retrospective cohort study (N = 102), and 1 retrospective subgroup analysis from a RCT (N = 581). The prospective study reported an association between Signatera test results at diagnosis, during chemotherapy treatment, and during surveillance following cystectomy to prognosis. The retrospective study reported an association between Signatera test results at diagnosis and during surveillance following cystectomy to prognosis; patients in this study did not receive chemotherapy. The retrospective subgroup analysis reported an association between test results and response to atezolizumab treatment. Study limitations, including a lack of comparison to tests used for the same purpose preclude drawing conclusions about clinical validity and usefulness. No study reported management changes made in response to ctDNA test results. There is no direct evidence that the use of the test improves health outcomes, and indirect evidence is not sufficient to draw conclusions about clinical validity.

Tumor-Informed Circulating Tumor DNA Testing with Signatera in Individuals with Non-Small Cell Lung Cancer

Clinical Context and Test Purpose

The purpose of Signatera testing in individuals with non-small cell lung cancer (NSCLC) is to predict disease course to inform treatment decisions and to monitor for recurrence following surgical resection.

The following PICO was used to select literature to inform this review.

Populations

The relevant population of interest is individuals with NSCLC, or those who have been treated for NSCLC and are being monitored for recurrence.

Interventions

The test being considered is ctDNA testing with Signatera following surgical resection, to identify metastatic relapse at an early time point, and aid in the selection of individuals who may benefit from early/adjuvant treatment.

Adjuvant platinum-based chemotherapy is not the standard of care following surgery for NSCLC; treatment improves cure rates after surgery in only 5% of patients, and 20% of patients receiving chemotherapy experience acute toxicities. Signatera testing is proposed to select patients who are very likely to relapse post-operatively and who might benefit from adjuvant treatment.

Comparators

Radiographic imaging is used for disease monitoring in individuals with NSCLC. Detection of relapse and monitoring of response to treatment in the metastatic setting is performed by standard computed tomography scan, with frequency and type of imaging depending on primary treatment and stage. For patients with stage I-II NSCLC following completion of definitive therapy, NCCN guidelines recommend history and physical and chest computed tomography every 6 months for 2 to 3 years, then annually. For patients with primary treatment that included radiotherapy, surveillance is recommended every 3 to 6 months for 3 years, and every 6 months for 2 years, then annually.

Treatment options following recurrence include resection and/or systemic therapy.

Outcomes

The general outcomes of interest are disease-specific survival, test accuracy and validity, and change in disease status. Specific outcomes of interest are recurrence risk, RFS, and overall survival at follow-up.

Beneficial outcomes of a true positive test would be an individual undergoing potentially beneficial additional treatment such as chemotherapy at an earlier time point than if a relapse were identified clinically.

Harmful outcomes of a false positive test would be undergoing unnecessary or incorrect treatment, and experiencing adverse effects of such treatment.

Nonrandomized Trial

The evidence for the use of Signatera to detect relapse in NSCLC following surgery is limited to a subgroup analysis of 24 individuals enrolled in TRACERx, a longitudinal cohort study of tumor sampling and genetic analysis in individuals with NSCLC.[14,] Of 14 individuals with confirmed relapse, 13 (93%) had a positive ctDNA test (defined as at least 2 single-nucleotide variants detected). Of 10 individuals with no relapse after a median follow up of 775 days, (range 688 to 945 days), 1 had a positive ctDNA test (10%).

Study limitations are shown in Tables 15 and 16. Major limitations include no comparison to standard surveillance methods and imprecise estimates due to the small sample size. Additionally, the commercially available Signatera has been updated since this publication.

Table 14. Nonrandomized Study of Signatera Testing in Non-Small Cell Lung Cancer

Study	Study Population	Study Design and Setting	Reference Standard	Threshold for Positive Index Test	Timing of Reference and Index Tests	Blinding of Assessors	Main Results
Abbosh et al (2017)[14,]	24 individuals with early-stage NSCLC	Prospective, subgroup of patients enrolled in the TRACERx Study	Clinical assessment and chest radiograph	Greater or equal to 2 variants detected out of 16	Every 3 months for 2 years, then every 6 months thereafter; individuals were followed up for a median of 775 days	Yes	Of 14 individuals with confirmed relapse, 13 (93%) had a positive ctDNA test. Of 10 individuals with no relapse after a median follow up of 775 days (range 688 to 945 days), 1 had a positive ctDNA test (10%). Median interval between ctDNA detection and NSCLC relapse confirmed by CT imaging indicated by clinical and chest radiograph follow-up (lead time) was 70 days (range, 10 to 346 days).

CT: computed tomography; ctDNA: circulating tumor DNA; NSCLC: non small cell lung cancer.

Table 15. Study Relevance Limitations

Study	Population[a]	Intervention[b]	Comparator[c]	Outcomes[d]	Duration of Follow-Up[e]
Abbosh et al (2017)[14,]			3. No comparison to standard methods of monitoring for relapse	1. Health outcomes not assessed	

The study limitations stated in this table are those notable in the current review; this is not a comprehensive gaps assessment.
[a] Population key: 1. Intended use population unclear; 2. Clinical context is unclear; 3. Study population is unclear; 4. Study population not representative of intended use.
[b] Intervention key: 1. Classification thresholds not defined; 2. Version used unclear; 3. Not intervention of interest.
[c] Comparator key: 1. Classification thresholds not defined; 2. Not compared to credible reference standard; 3. Not compared to other tests in use for same purpose.
[d] Outcomes key: 1. Study does not directly assess a key health outcome; 2. Evidence chain or decision model not explicated; 3. Key clinical validity outcomes not reported (sensitivity, specificity and predictive values); 4. Reclassification of diagnostic or risk categories not reported; 5. Adverse events of the test not described (excluding minor discomforts and inconvenience of venipuncture or noninvasive tests).
[e] Follow-Up key: 1. Follow-up duration not sufficient with respect to natural history of disease (true positives, true negatives, false positives, false negatives cannot be determined).

Table 16. Study Design and Conduct Limitations

Study	Selection[a]	Blinding[b]	Delivery of Test[c]	Selective Reporting[d]	Data Completeness[e]	Statistical[f]
Abbosh et al (2017)[14,]	2. Subgroup analysis, subset of the first 100 participants enrolled in the study; unclear if selection was consecutive		2. Timing of ctDNA testing unclear			1. No comparison to imaging, no confidence intervals

ctDNA: circulating tumor DNA.
The study limitations stated in this table are those notable in the current review; this is not a comprehensive gaps assessment.
[a] Selection key: 1. Selection not described; 2. Selection not random or consecutive (i.e., convenience).
[b] Blinding key: 1. Not blinded to results of reference or other comparator tests.
[c] Test Delivery key: 1. Timing of delivery of index or reference test not described; 2. Timing of index and comparator tests not same; 3. Procedure for interpreting tests not described; 4. Expertise of evaluators not described.
[d] Selective Reporting key: 1. Not registered; 2. Evidence of selective reporting; 3. Evidence of selective publication.
[e] Data Completeness key: 1. Inadequate description of indeterminate and missing samples; 2. High number of samples excluded; 3. High loss to follow-up or missing data.
[f] Statistical key: 1. Confidence intervals and/or p values not reported; 2. Comparison to other tests not reported.

Section Summary: Tumor-Informed Circulating Tumor DNA Testing with Signatera in Individuals with Non-Small Cell Lung Cancer

For individuals with NSCLC who receive tumor-informed ctDNA testing with Signatera to guide treatment decisions and monitor for recurrence, the evidence includes 1 subgroup analysis of participants enrolled in a prospective observational study (N = 24). Of 14 individuals with confirmed relapse, 13 (93%) had a positive ctDNA test (defined as at least 2 single-nucleotide variants detected). Of 10 individuals with no relapse after a median follow up of 775 days, (range 688 to 945 days), 1 had a positive ctDNA test (10%). This study's small sample size and lack of a comparator preclude drawing conclusions about clinical validity. There is no direct evidence that the use of the test improves health outcomes, and indirect evidence is not sufficient to draw conclusions about clinical validity.

Tumor-Informed Circulating Tumor DNA Testing with Signatera in Individuals with Esophageal Cancer

Clinical Context and Test Purpose

The purpose of Signatera testing in individuals with esophageal cancer is to detect minimal residual disease following surgical resection and to monitor for disease recurrence.

The following PICO was used to select literature to inform this review.

Populations

The relevant population of interest is individuals with esophageal cancer who have undergone surgical resection.

Interventions

The test being considered is ctDNA testing with Signatera:

- Following surgical resection, to detect minimal residual disease and aid in the selection of individuals who may benefit from early/adjuvant treatment, or

- For disease monitoring after curative treatment, to identify metastatic relapse at an early time point, and aid in the selection of individuals who may benefit from early/adjuvant treatment.

Comparators
Recommendations on surveillance and monitoring following esophageal cancer treatment include periodic upper endoscopy, laboratory tests, and imaging as indicated. Specific recommendations depend on tumor classification.

Outcomes
The general outcomes of interest are disease-specific survival, test accuracy and validity, and change in disease status. Specific outcomes of interest are recurrence risk, RFS, and overall survival at follow-up.

Beneficial outcomes of a true positive test would be an individual undergoing potentially beneficial additional treatment at an earlier time point than if a relapse were identified clinically.
Harmful outcomes of a false positive test would be undergoing unnecessary or incorrect treatment and experiencing adverse effects of such treatment.

Study Selection Criteria
For the evaluation of clinical validity of the Signatera test, studies that meet the following eligibility criteria were considered:

- Reported on the accuracy of the marketed version of the technology
- Included a suitable reference standard
- Individual/sample clinical characteristics were described
- Individual/sample selection criteria were described.

Nonrandomized Trial
One noncomparative retrospective study reported the association of Signatera testing measured before and after surgery with relapse and recurrence in 17 individuals with esophageal adenocarcinoma (Tables 17 and 18). Patients who were ctDNA-positive before surgery had significantly poorer DFS (p<.042), with a median DFS of 32.0 months vs. 63.0 months in ctDNA-negative preoperative patients. This study was limited by the very small number sample size, and its retrospective design (Tables 19 and 20).

Table 17. Nonrandomized Study of Signatera Testing to Predict Relapse in Esophageal Cancer - Study Characteristics

Study	Study Population	Study Design and Setting	Reference Standard	Threshold for Positive Index Test	Timing of Reference and Index Tests	Blinding of Assessors
Ococks et al (2021)[15,]	17 individuals with esophageal adenocarcinoma who had undergone surgery	Retrospective	Radiological imaging	2 or more variants detected out of 16	Blood samples were collected before and after surgical treatment and patients were followed up for a median of 43.4 months.	Yes

Table 18. Recurrence Rates by Risk Category in Nonrandomized Studies of Signatera in Resected Esophageal Cancer

Study	Median DFS		
	ctDNA Positive	ctDNA Negative	p for comparison
Ococks et al (2021)[15],			
ctDNA status before surgery			
Recurrence rate	5/11	0/6	
Median DFS	32.0 months	63.0 months	.042
ctDNA status following surgery			
Recurrence rate	4/4	1/13	NR
Median DFS	14.2 months	51.2 months	NR

ctDNA: circulating tumor DNA; DFS: disease-free surival; NR: not reported.

Table 19. Study Relevance Limitations

Study	Population[a]	Intervention[b]	Comparator[c]	Outcomes[d]	Duration of Follow-Up[e]
Ococks et al (2021)[15],		2. Unclear if the test used was the commercially available version	3. No comparison to tests used for the same purpose		

The study limitations stated in this table are those notable in the current review; this is not a comprehensive gaps assessment.
[a] Population key: 1. Intended use population unclear; 2. Clinical context is unclear; 3. Study population is unclear; 4. Study population not representative of intended use.
[b] Intervention key: 1. Classification thresholds not defined; 2. Version used unclear; 3. Not intervention of interest.
[c] Comparator key: 1. Classification thresholds not defined; 2. Not compared to credible reference standard; 3. Not compared to other tests in use for same purpose.
[d] Outcomes key: 1. Study does not directly assess a key health outcome; 2. Evidence chain or decision model not explicated; 3. Key clinical validity outcomes not reported (sensitivity, specificity and predictive values); 4. Reclassification of diagnostic or risk categories not reported; 5. Adverse events of the test not described (excluding minor discomforts and inconvenience of venipuncture or noninvasive tests).
[e] Follow-Up key: 1. Follow-up duration not sufficient with respect to natural history of disease (true positives, true negatives, false positives, false negatives cannot be determined).

Table 20. Study Design and Conduct Limitations

Study	Selection[a]	Blinding[b]	Delivery of Test[c]	Selective Reporting[d]	Data Completeness[e]	Statistical[f]
Ococks et al (2021)[15],					Excluded individuals who did not undergo surgery	Imprecise estimates due to small sample size

The study limitations stated in this table are those notable in the current review; this is not a comprehensive gaps assessment.
[a] Selection key: 1. Selection not described; 2. Selection not random or consecutive (i.e., convenience).
[b] Blinding key: 1. Not blinded to results of reference or other comparator tests.
[c] Test Delivery key: 1. Timing of delivery of index or reference test not described; 2. Timing of index and comparator tests not same; 3. Procedure for interpreting tests not described; 4. Expertise of evaluators not described.
[d] Selective Reporting key: 1. Not registered; 2. Evidence of selective reporting; 3. Evidence of selective publication.
[e] Data Completeness key: 1. Inadequate description of indeterminate and missing samples; 2. High number of samples excluded; 3. High loss to follow-up or missing data.
[f] Statistical key: 1. Confidence intervals and/or p values not reported; 2. Comparison to other tests not reported.

Section Summary: Tumor-Informed Circulating Tumor DNA Testing with Signatera in Individuals with Esophageal Cancer

For individuals with esophageal cancer who receive tumor-informed ctDNA testing with Signatera to guide treatment decisions and monitor for recurrence, the evidence includes 1 noncomparative, retrospective study (N = 17). Patients who were ctDNA-positive before surgery had significantly

poorer DFS (p<.042), with a median DFS of 32.0 months versus 63.0 months in ctDNA-negative preoperative patients. This study was limited by its small number sample size and retrospective design. There is no direct evidence that the use of the test improves health outcomes. Due to the study's limitations and lack of additional supporting studies, the evidence is not sufficient to draw conclusions on clinical validity. Additionally, the management pathway for Signatera testing in esophageal cancer has not been clearly defined.

Tumor-Informed Circulating Tumor DNA Testing with Signatera in Individuals with Solid Tumors Receiving Immunotherapy

Clinical Context and Test Purpose

The purpose of Signatera testing in individuals with solid tumors who have received immunotherapy is to monitor treatment response and inform subsequent treatment decisions. Signatera is proposed as a method to stratify patients according to their likelihood of response to immunotherapy, to guide treatment decisions.

The following PICO was used to select literature to inform this review.

Populations

The relevant population of interest is individuals with solid tumors who have received immune checkpoint inhibitor (ICI) therapy.

Interventions

The test being considered is ctDNA testing with Signatera.

Comparators

For individuals with solid tumors receiving immunotherapy, treatment response is monitored by repeated radiographic evaluation of the tumor.

Outcomes

The general outcomes of interest are disease-specific survival, test accuracy and validity, and change in disease status. Specific outcomes of interest are recurrence risk, RFS, and overall survival at follow-up.

If the test is used to *rule-in* individuals with solid tumors who are likely to respond to immunotherapy, the performance characteristics of most interest are positive predictive value and specificity.
If the test is used to *rule-out* individuals with solid tumors who are unlikely to respond to immunotherapy, the performance characteristics of most interest are negative predictive value and sensitivity.

Study Selection Criteria

For the evaluation of clinical validity of the Signatera test, studies that meet the following eligibility criteria were considered:

- Reported on the accuracy of the marketed version of the technology
- Included a suitable reference standard
- Individual/sample clinical characteristics were described
- Individual/sample selection criteria were described.

Nonrandomized Trial

Bratman et al (2020) evaluated Signatera to predict treatment response in 106 individuals receiving pembrolizumab for solid tumors, including squamous cell cancer of head and neck, triple negative breast cancer, high-grade serous ovarian cancer, malignant melanoma, and mixed solid tumors (Tables 21 and 22).[16,

Lower-than-median ctDNA levels at baseline were associated with improved overall survival (adjusted HR , 0.49; 95% CI, 0.29 to 0.83) and progression-free survival (PFS) (adjusted HR, 0.54; 95% CI, 0.34 to 0.85). Among participants with at least 2 ctDNA measurements, any rise in ctDNA levels during surveillance above baseline was associated with rapid disease progression and poor survival (median overall survival, 13.7 months), whereas among 12 patients whose ctDNA cleared during treatment, overall survival was 100% at a median follow up of 25.4 months (range, 10.8 to 29.5 months) following the first clearance.

Study limitations are shown in Tables 23 and 24. This single-center study is limited by its small sample size and variability in results across different tumor types. The study did not include a comparison of monitoring with ctDNA to standard methods of monitoring response such as repeat imaging.

Table 21. Nonrandomized Study of Signatera Testing to Predict Response to Immunotherapy in Individuals with Solid Tumors - Study Characteristics

Study	Study Population	Study Design and Setting	Reference Standard	Threshold for Positive Index Test	Timing of Reference and Index Tests	Blinding of Assessors
Bratman et al (2020)[16,]	106 individuals with advanced solid tumors who were enrolled in a Phase II clinical trial of pembrolizumab (NCT02644369)	Prospective, single center	TMB, PD-L1 testing, radiological imaging	Greater or equal to 2 variants detected out of 16	Baseline sample obtained and after every 3 cycles; individuals were followed up for a median of 25 months	Yes

PD-L1: programmed death ligand-1; TMB: tumor mutational burden.

Table 22. Overall Survival by Risk Category in a Nonrandomized Study of Signatera to Monitor Response to Immunotherapy

	Overall Survival
Bratman et al (2020)[16,]	
Lower than median ctDNA at baseline	adjusted HR, 0.49 (95% CI, 0.29 to 0.83)
ctDNA increased (n = 45)	13.7 months
ctDNA decreased but still detectable (n = 16)	23.8 months
ctDNA cleared (n = 12)	25.4 months (range 10.8 to 29.5 months)

CI: confidence interval; ctDNA: circulating tumor DNA; HR: hazard ratio.

Table 23. Study Relevance Limitations

Study	Population[a]	Intervention[b]	Comparator[c]	Outcomes[d]	Duration of Follow-Up[e]
Bratman et al (2020)[16,]	1, 2. Unclear what management changes would be implemented based on test results.		No comparison to standard surveillance methods	3. Clinical validity outcomes not reported	

The study limitations stated in this table are those notable in the current review; this is not a comprehensive gaps assessment.
[a] Population key: 1. Intended use population unclear; 2. Clinical context is unclear; 3. Study population is unclear; 4. Study population not representative of intended use.
[b] Intervention key: 1. Classification thresholds not defined; 2. Version used unclear; 3. Not intervention of interest.
[c] Comparator key: 1. Classification thresholds not defined; 2. Not compared to credible reference standard; 3. Not compared to other tests in use for same purpose.

[d] Outcomes key: 1. Study does not directly assess a key health outcome; 2. Evidence chain or decision model not explicated; 3. Key clinical validity outcomes not reported (sensitivity, specificity and predictive values); 4. Reclassification of diagnostic or risk categories not reported; 5. Adverse events of the test not described (excluding minor discomforts and inconvenience of venipuncture or noninvasive tests).
[e] Follow-Up key: 1. Follow-up duration not sufficient with respect to natural history of disease (true positives, true negatives, false positives, false negatives cannot be determined).

Table 24. Study Design and Conduct Limitations

Study	Selection[a]	Blinding[b]	Delivery of Test[c]	Selective Reporting[d]	Data Completeness[e]	Statistical[f]
Bratman et al (2020)[16,]						2. Comparison to other tests not reported

The study limitations stated in this table are those notable in the current review; this is not a comprehensive gaps assessment.
[a] Selection key: 1. Selection not described; 2. Selection not random or consecutive (i.e., convenience).
[b] Blinding key: 1. Not blinded to results of reference or other comparator tests.
[c] Test Delivery key: 1. Timing of delivery of index or reference test not described; 2. Timing of index and comparator tests not same; 3. Procedure for interpreting tests not described; 4. Expertise of evaluators not described.
[d] Selective Reporting key: 1. Not registered; 2. Evidence of selective reporting; 3. Evidence of selective publication.
[e] Data Completeness key: 1. Inadequate description of indeterminate and missing samples; 2. High number of samples excluded; 3. High loss to follow-up or missing data.
[f] Statistical key: 1. Confidence intervals and/or p values not reported; 2. Comparison to other tests not reported.

Section Summary: Tumor-Informed Circulating Tumor DNA Testing with Signatera in Individuals with Solid Tumors Receiving Immunotherapy

For individuals with solid tumors who receive tumor-informed ctDNA testing with Signatera to monitor response to immunotherapy, the evidence includes a subgroup analysis of individuals enrolled in a nonrandomized trial of pembrolizumab (N = 106). The subgroup analysis evaluated Signatera testing to monitor response to immunotherapy in individuals with advanced solid tumors who were enrolled in a Phase II clinical trial of pembrolizumab. Lower-than-median ctDNA levels at baseline were associated with improved overall survival (adjusted HR, 0.49; 95% CI, 0.29 to 0.83) and PFS (adjusted HR, 0.54; 95% CI, 0.34 to 0.85). The study was limited by a small sample size, variability in results across different tumor types, and lack of a comparison to standard methods of monitoring response to treatment. There is no direct evidence that the use of the test improves health outcomes, and indirect evidence is not sufficient to draw conclusions about clinical validity. Additionally, the management pathway for Signatera testing for monitoring response to immunotherapy has not been clearly defined.

Supplemental Information

The purpose of the following information is to provide reference material. Inclusion does not imply endorsement or alignment with the evidence review conclusions.

Practice Guidelines and Position Statements

Guidelines or position statements will be considered for inclusion in 'Supplemental Information' if they were issued by, or jointly by, a US professional society, an international society with US representation, or National Institute for Health and Care Excellence (NICE). Priority will be given to guidelines that are informed by a systematic review, include strength of evidence ratings, and include a description of management of conflict of interest.

American Society of Clinical Oncology

The American Society of Clinical Oncology (ASCO) 2022 guideline update on biomarkers for systemic therapy in metastatic breast cancer (MBC) does not recommend the use of circulating tumor DNA (ctDNA) as a biomarker to monitor the response to therapy (Type of recommendation: informal

consensus-based; Quality of evidence: low; Strength of recommendation: moderate). The guidelines also provide the following recommendations:

- "Patients with locally recurrent unresectable or metastatic hormone receptor-positive and human epidermal growth factor receptor 2 (HER2)-negative breast cancer who are candidates for a treatment regimen that includes a phosphatidylinositol 3-kinase inhibitor and hormonal therapy should undergo testing for PIK3CA mutations using next-generation sequencing of tumor tissue or circulating tumor DNA (ctDNA) in plasma to determine their eligibility for treatment with the phosphatidylinositol 3-kinase inhibitor alpelisib plus fulvestrant. If no mutation is found in ctDNA, testing in tumor tissue, if available, should be used as this will detect a small number of additional patients with PIK3CA mutations (Type: evidence-based, benefits outweigh harms; Evidence quality: high; Strength of recommendation: strong)."
- "There are insufficient data at present to recommend routine testing for ESR1 mutations to guide therapy for hormone receptor-positive, HER2-negative MBC. Existing data suggest reduced efficacy of aromatase inhibitors (AIs) compared with the selective estrogen receptor degrader fulvestrant in patients who have tumor or ctDNA with ESR1 mutations (Type: informal consensus; Evidence quality: insufficient; Strength of recommendation: moderate)."
- "There are insufficient data to recommend routine use of ctDNA to monitor response to therapy among patients with MBC (Type: informal consensus; Evidence quality: low; Strength of recommendation: moderate)."

National Comprehensive Cancer Network

National Comprehensive Cancer Network (NCCN) guidelines either do not specifically address tumor-informed ctDNA testing for the cancer types included in this review, or do not provide specific recommendations for use.

The guideline on colon cancer states: "The panel believes that there are insufficient data to recommend the use of...post-surgical ctDNA to estimate risk of recurrence or determine adjuvant therapy...The NCCN Panel encourages enrollment in clinical trials to help with the generation of additional data on these assays."[17,]

The guideline on breast cancer states that for recurrent/stage IV disease: "Tissue or plasma-based circulating tumor DNA (ctDNA) assays may be used and each of these have benefits and limitations for diagnosis and disease progression. Tissue-based assays have greater sensitivity, but ctDNA may reflect tumor heterogeneity more accurately. If one specimen is negative for actionable biomarkers, testing on the alternative specimen can be considered." Additionally, "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 relevant discussion for these recommendations is pending an update.[18,]
The guideline on esophageal and esophagogastric junction cancers states: "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, particularly those who are unable to have a clinical biopsy for disease surveillance and management. The detection of mutations/alterations in DNA shed from 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 who may be unable to undergo a traditional biopsy or for disease progression monitoring, 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."[19,]

The guideline on non-small cell lung cancer (NSCLC) states the following in their section on molecular and biomarker analysis:[20,]

- "ctDNA testing should not be used in lieu of a histologic tissue diagnosis.
- ctDNA is not routinely recommended in settings other than advanced/metastatic disease. For stages I–III, tissue-based testing is preferred. Metastatic disease confined to the thorax may have a higher yield with tissue-based testing.
- Studies have demonstrated ctDNA and tissue testing to have very high specificity. Both ctDNA and tissue testing have appreciable false-negative rates, supporting the complementarity of these approaches, and data support complementary testing to reduce turnaround time and increase yield of targetable alteration detection.
- Limitations of ctDNA testing that can impact interpretation include:
    - Low tumor fraction/ctDNA; some assays include a measure of ctDNA fraction, which can aid in identification of situations in which low ctDNA fraction might suggest compromised sensitivity.
    - The presence of mutations from sites other than the target lesion, most commonly clonal hematopoiesis of indeterminate potential (CHIP) or post-chemotherapy marrow clones. KRAS and TP53 can be seen in either of these circumstances
    - The inherent ability of the assay to detect fusions or other genomic variation of relevance."

U.S. Preventive Services Task Force Recommendations
Not applicable.

Medicare National and Local Coverage
There is no national coverage determination. In the absence of a national coverage determination, coverage decisions are left to the discretion of local Medicare carriers.

Local coverage guidance for California is provided by the provided by the Molecular Diagnostic Services Program (MolDx) in the document MolDX: Minimal Residual Disease Testing for Cancer along with the related Billing and Coding: MolDX: Minimal Residual Disease Testing for Solid Tumor Cancers.

MolDx provides limited coverage for MRD testing in cancer when **ALL of the following are true:**

1. If Next-Generation Sequencing (NGS) methodology is used in testing, the conditions set by NCD 90.2 are fulfilled (summarized: the patient has advanced cancer; plans on being treated for said cancer, and has not been previously tested with the same test for the same genetic content) or are not applicable (the patient does not have cancer as defined below);
2. The patient has a personal history of cancer, the type and staging of which is within the intended use of the MRD test;
3. The identification of recurrence or progression of disease within the intended use population of the test is identified in the National Comprehensive Cancer Network (NCCN) or other established guidelines as a condition that requires a definitive change in patient management;
4. The test is demonstrated to identify molecular recurrence or progression before there is clinical, biological or radiographical evidence of recurrence or progression AND demonstrates sensitivity and specificity of subsequent recurrence or progression comparable with or superior to radiographical or other evidence (as per the standard-of-care for monitoring a given cancer type) of recurrence or progression;
5. To be reasonable and necessary, it must also be medically acceptable that the test being utilized precludes other surveillance or monitoring tests intended to provide the same or similar information, unless they either (a) are required to follow-up or confirm the findings of this test or (b) are medically required for further assessment and management of the patient;

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

Intended uses that have met clinical validity (CV) criteria under the policy include:

(1) the diagnosis of disease progression, recurrence, or relapse for advanced colorectal (Signatera, Guardant Reveal, and Oncodetect), muscle-invasive bladder, ovarian and (neoadjuvant) breast cancers (Signatera), and HPV-negative head and neck squamous cell carcinoma (RaDaR)

(2) the diagnosis of disease recurrence or relapse in patients with a personal history of cancer for non-small cell lung cancer (NSCLC) (Signatera), advanced breast (RaDaR and Signatera) and HPV-driven oropharyngeal cancers (NavDX)

(3) the monitoring of response to immune-checkpoint inhibitor therapy for colorectal cancer (Guardant Response) or any solid tumor (Signatera). However, the tests listed in the table may have only been approved for one or more (but not necessarily all) of these indications.

The following tests have met the MolDx criteria for coverage under the policy:

Cancers with Indicated Use	Test
Breast Cancer Colorectal cancer Muscle-invasive bladder cancer Non-small cell lung cancer Ovarian cancer	Signatera Bespoke Assay Design (by comprehensive genomic profile (CGP)) + Plasma Series Bundle for Molecular Residual Disease (Natera, Inc) Signatera *Recurrence Monitoring* Bespoke Assay Design + single Plasma Test (Natera, Inc) Signatera *Recurrence Monitoring* Plasma Test Bundle (Natera, Inc) Signatera *Recurrence Monitoring* single Plasma Test (Natera, Inc)
HPV-negative head and neck squamous cell carcinoma	RaDaR Bespoke Assay Design + Plasma Series Bundle for Molecular Residual Disease (NeoGenomics Laboratories, Inc) RaDaR *Recurrence Monitoring* Bespoke Assay Design + single Plasma Test (NeoGenomics Laboratories, Inc) RaDaR *Recurrence Monitoring* single Plasma Test (NeoGenomics Laboratories, Inc)
Colorectal cancer	Guardant Reveal MRD Bundle (Guardant, Inc) Guardant360 Response (Guardant, Inc) Guardant Reveal single Plasma Test (Guardant, Inc)
Colorectal cancer	Oncodetect Bespoke Assay Design + single Plasma Test (Exact Sciences Corp) Oncodetect Plasma Test Bundle (Exact Sciences Corp) Oncodetect single Plasma Test (Exact Sciences Corp)
HPV-driven oropharyngeal cancers	NavDX single Plasma Test (Naveris, Inc)

Ongoing and Unpublished Clinical Trials

Some currently unpublished trials that might influence this review are listed in Table 25.

Table 25. Summary of Key Trials

NCT No.	Trial Name	Planned Enrollment	Completion Date
Ongoing			
NCT06450314	Decreasing Treatment for Metastatic HER2-Positive Breast Cancer With Undectable Cancer Levels in Blood Tests. (HEROES)	170	Nov 2029
NCT05212779	Predicting the Risk of Ovarian Cancer Recurrence Using Circulating Tumor DNA to Assess Residual Disease	45	Dec 2024
NCT04761783[a]	BESPOKE Study of ctDNA Guided Immunotherapy	1539	May 2024
NCT04264702[a]	BESPOKE Study of ctDNA Guided Therapy in Colorectal Cancer	2000	May 2025
NCT04786600[a]	A Phase II Randomized Therapeutic Optimization Trial for Subjects With Refractory Metastatic Colorectal Cancer Using ctDNA: Rapid 1 Trial	78	Mach 2024
NCT05178576[a]	A Single Arm Phase II Study to Evaluate Treatment With Gevokizumab in individuals With Stage II/III Colon Cancer Who Are ctDNA-positive After Curative Surgery and Adjuvant Chemotherapy	31	Feb 2027
NCT04920032[a]	Proof of Concept Study of ctDNA Guided Change in Treatment for Refractory Minimal Residual Disease in Colon Adenocarcinomas	22	Dec 2025
NCT05060003[a]	A Phase II Randomized Study of Tiragolumab Plus Atezolizumab Versus Atezolizumab in the Treatment of Stage II Melanoma individuals Who Are ctDNA-positive Following Resection	244	Nov 2023 (terminated)
NCT05081024[a]	Establishing a ctDNA Biomarker to Improve Organ Preserving Strategies in individuals With Rectal Cancer	50	Sep 2024
NCT05067842	A Pilot Observational Study to Assess Feasibility of Tumor Response Assessment by Circulating Tumor DNA (ctDNA) in individuals With Locally Advanced Esophageal and GE Junction Adenocarcinoma Undergoing Treatment With Total Upfront Chemotherapy and Chemoradiation	30	Apr 2028
NCT04670588	A Prospective Observational Study to Determine the Feasibility of Tumor Response Assessment by Circulating Tumor DNA in individuals With Locally Advanced Rectal Cancer Undergoing Total Neoadjuvant Therapy	30	June 2022 (withdrawn)
NCT04929015	Peritoneal Carcinomatosis Leveraging ctDNA Guided Treatment in GI Cancer Study (PERICLES Study)	30	Nov 2024
NCT05058183[a]	Safe De-escalation of Chemotherapy for Stage 1 Breast Cancer	400	Dec 2028
NCT05174169[a]	Colon Adjuvant Chemotherapy Based on Evaluation of Residual Disease	1912	Mar 2030
NCT05757843	Using Circulating Tumor DNA to Personalize Duration of Consolidation Durvalumab	56	Dec 2025
NCT05965479	Developing ctDNA Guided Adjuvant Therapy for Gastrooesophageal Cancer (DECIPHER)	25	Apr 2028
NCT05914792	Longitudinal ctDNA Surveillance for Older Women With ER+ Breast Cancer Who Omit Surgery	40	Jun 2030

NCT: national clinical trial.
[a] Denotes industry-sponsored or cosponsored trial.

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Documentation for Clinical Review

Please provide the following documentation:

- History and physical and/or consultation notes including:
  - Clinical findings (i.e., pertinent symptoms and duration)
  - Activity and functional limitations
  - Family history, if applicable
  - Reason for procedure/test/device, when applicable (e.g., routine screening, suspected recurrence or progression, etc.)
  - Pertinent past procedural and surgical history
  - Past and present diagnostic testing and results, including previous MRD testing
  - Prior treatments, duration, and response
  - Treatment plan (i.e., surgical intervention)
- Radiology report(s) and interpretation (i.e., MRI, CT, PET)

Post Service (in addition to the above, please include the following):

- Results/reports of tests performed
- Procedure report(s)

Coding

The list of codes in this Medical Policy is intended as a general reference and may not cover all codes. Inclusion or exclusion of a code(s) does not constitute or imply member coverage or provider reimbursement policy.

Type	Code	Description
CPT®	0306U	Oncology (minimal residual disease [MRD]), next-generation targeted sequencing analysis, cell-free DNA, initial (baseline) assessment to determine a patient-specific panel for future comparisons to evaluate for MRD
	0307U	Oncology (minimal residual disease [MRD]), next-generation targeted sequencing analysis of a patient-specific panel, cell-free DNA, subsequent assessment with comparison to previously analyzed patient specimens to evaluate for MRD
	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
HCPCS	None	

Policy History

This section provides a chronological history of the activities, updates and changes that have occurred with this Medical Policy.

Effective Date	Action
06/01/2022	New policy.
11/01/2022	Coding update
03/01/2023	Annual update. Converted to custom policy. Policy statement, guidelines and literature updated.
10/01/2025	Policy reactivated. Previously archived from 07/01/2023 to 09/30/2025

Definitions of Decision Determinations

Healthcare Services: For the purpose of this Medical Policy, Healthcare Services means procedures, treatments, supplies, devices, and equipment.

Medically Necessary: Healthcare Services that are Medically Necessary include only those which have been established as safe and effective, are furnished under generally accepted professional standards to treat illness, injury or medical condition, and which, as determined by Blue Shield of California, are: (a) consistent with Blue Shield of California medical policy; (b) consistent with the symptoms or diagnosis; (c) not furnished primarily for the convenience of the patient, the attending Physician or other provider; (d) furnished at the most appropriate level which can be provided safely and effectively to the member; and (e) not more costly than an alternative service or sequence of services at least as likely to produce equivalent therapeutic or diagnostic results as to the diagnosis or treatment of the member's illness, injury, or disease.

Investigational or Experimental: Healthcare Services which do not meet ALL of the following five (5) elements are considered investigational or experimental:

A. The technology must have final approval from the appropriate government regulatory bodies.
   - This criterion applies to drugs, biological products, devices and any other product or procedure that must have final approval to market from the U.S. Food and Drug Administration ("FDA") or any other federal governmental body with authority to regulate the use of the technology.
   - Any approval that is granted as an interim step in the FDA's or any other federal governmental body's regulatory process is not sufficient.
   - The indications for which the technology is approved need not be the same as those which Blue Shield of California is evaluating.

B. The scientific evidence must permit conclusions concerning the effect of the technology on health outcomes.
   - The evidence should consist of well-designed and well-conducted investigations published in peer-reviewed journals. The quality of the body of studies and the consistency of the results are considered in evaluating the evidence.
   - The evidence should demonstrate that the technology can measure or alter the physiological changes related to a disease, injury, illness, or condition. In addition, there should be evidence, or a convincing argument based on established medical facts that such measurement or alteration affects health outcomes.

C. The technology must improve the net health outcome.
   - The technology's beneficial effects on health outcomes should outweigh any harmful effects on health outcomes.

D. The technology must be as beneficial as any established alternatives.

- The technology should improve the net health outcome as much as, or more than, established alternatives.

E. The improvement must be attainable outside the investigational setting.
- When used under the usual conditions of medical practice, the technology should be reasonably expected to satisfy Criteria C and D.

Feedback

Blue Shield of California is interested in receiving feedback relative to developing, adopting, and reviewing criteria for medical policy. Any licensed practitioner who is contracted with Blue Shield of California or Blue Shield of California Promise Health Plan is welcome to provide comments, suggestions, or concerns. Our internal policy committees will receive and take your comments into consideration. Our medical policies are available to view or download at www.blueshieldca.com/provider.

For medical policy feedback, please send comments to: MedPolicy@blueshieldca.com

Questions regarding the applicability of this policy should be directed to the Prior Authorization Department at (800) 541-6652, or the Transplant Case Management Department at (800) 637-2066 ext. 3507708 or visit the provider portal at www.blueshieldca.com/provider.

Disclaimer: Blue Shield of California may consider published peer-reviewed scientific literature, national guidelines, and local standards of practice in developing its medical policy. Federal and state law, as well as member health services contract language, including definitions and specific contract provisions/exclusions, take precedence over medical policy and must be considered first in determining covered services. Member health services contracts may differ in their benefits. Blue Shield reserves the right to review and update policies as appropriate.

Appendix A

POLICY STATEMENT	
BEFORE	**AFTER** **Blue font: Verbiage Changes/Additions**
Reactivated Policy **Policy Statement:** N/A	**Tumor-Informed Circulating Tumor DNA Testing for Cancer Management 2.04.153** **Policy Statement:** I. Tumor-informed circulating tumor DNA testing (e.g., Signatera) is considered **investigational** for all indications. Note: For individuals enrolled in health plans subject to the Biomarker Testing Law (Health & Safety Code Section 1367.667 and the Insurance Code Section 10123.209), Centers for Medicare & Medicaid Services (CMS) Local Coverage Determination (LCD) may also apply. Please refer to the Medicare National and Local Coverage section of this policy and to MolDX: Minimal Residual Disease Testing for Cancer for reference.