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***Current Policy Effective Date: 11/1/26**
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Title: GT-Tumor-Informed Circulating Tumor DNA Testing for Cancer Management (e.g., Signatera™)

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 the disease course to inform treatment decisions and to monitor for recurrence following treatment. Unlike tumor-agnostic (tumor naïve) ctDNA assays (e.g. Guardant Reveal) which use a fixed, standardized panel of common cancer mutations without any prior knowledge of the tumor genomic profile, tumor informed ctDNA assay (e.g. Signatera) requires prior sequencing of the patient's primary tumor tissue to build a personalized assay to track patient-specific mutations in the plasma. Tumor-informed ctDNA assays generally offer higher sensitivity for minimal residual disease (MRD).

Medical Policy Statement

Tumor-informed circulating tumor DNA testing (e.g., Signatera) is established for muscle invasive bladder cancer (MIBC) when criteria are met.

Inclusionary and Exclusionary Guidelines

Inclusions:

Tumor-informed circulating tumor DNA testing (e.g., Signatera) for muscle invasive bladder cancer (MIBC) when **ALL** of the following criteria are met:

- Tumor is resectable
- Used as a predictive and prognostic biomarker after cystectomy
- Used to determine the use of adjuvant immunotherapy after cystectomy

Exclusions:

Tumor-informed circulating tumor DNA testing (e.g., Signatera) for all other diagnoses.

CPT/HCPCS Level II Codes

Established codes:

0340U

Other codes (investigational, not medically necessary, etc.):

N/A

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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.

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 systematic reviews, post-hoc and exploratory analysis of randomized studies, multiple noncomparative studies (N = 2,895), 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 reviews, post-hoc and exploratory analysis of randomized studies, and nonrandomized studies have reported an association between ctDNA results and prognosis when measured at diagnosis, following neoadjuvant therapy and surgery, during adjuvant therapy, and during surveillance after curative treatment, 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. There is insufficient evidence to determine that the technology results in an improvement in net health outcomes.

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 systematic reviews and 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. The systematic review included patients with early-stage triple-negative breast cancer and found that post-neoadjuvant (before or after surgery) ctDNA positivity was associated with a worse prognosis. 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. There is insufficient evidence to determine that the technology results in an improvement in net health outcomes.

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 randomized

controlled trial (RCT), 1 uncontrolled prospective cohort study (N = 68), 3 retrospective cohort studies (N = 381), and 2 retrospective subgroup analyses from randomized controlled trials (N = 581). 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 strongest evidence comes from IMvigor011, a phase 3, double-blind RCT. In this ctDNA-positive population, atezolizumab was associated with statistically significant improvements in disease-free survival and overall survival compared with placebo, supporting the predictive value of ctDNA positivity for identifying patients more likely to benefit from adjuvant immunotherapy. These results are further supported by exploratory analysis from IMvigor010. CheckMate 274, another phase III double blinded study which suggests that ctDNA-positive patients derive greater benefit from adjuvant immune checkpoint inhibition than ctDNA-negative patients. Overall, the current body of evidence indicates that ctDNA testing after cystectomy is a strong prognostic biomarker for recurrence risk in MIBC and has emerging predictive value for selecting patients for adjuvant immunotherapy. Five-year results confirm durable disease-free survival benefit and consistent safety profile for adjuvant nivolumab in high-risk MIUC. Linskrog et al (2023) presented a cohort study for ctDNA which was prognostic in NAC-treated and NAC-naïve patients with more than 5 years follow-up and studies indicated that the study outperformed pathologic downstaging in predicting treatment efficacy. The retrospective studies reported an association between Signatera test results at diagnosis and during surveillance following cystectomy to prognosis; patients received chemotherapy in 2 of these studies. There is sufficient evidence to determine that the technology results in an improvement in net health outcomes.

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 a systematic review, 2 subgroup analyses of participants enrolled in prospective observational studies. 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 demonstrated in a subgroup analysis that tumor-informed assays demonstrated higher specificity at landmark (0.97 vs. 0.93) and AUC (0.81 vs. 0.70) than tumor-agnostic approaches, while differences narrowed in longitudinal monitoring. 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. There is insufficient evidence to determine that the technology results in an improvement in net health outcomes.

For individuals with esophageal cancer who receive tumor-informed ctDNA testing with Signatera to guide treatment decisions and monitor for recurrence, the evidence includes a systematic review, a noncomparative, prospective study (N = 22), and 1 noncomparative, retrospective study (N = 17). 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 demonstrated that in a multivariate analysis, ctDNA positivity was associated with poorer progression-free survival (PFS) at baseline, after neoadjuvant therapy,

and during follow-up. In the prospective study, clearance of ctDNA post-surgery was associated with improved overall survival (median, 25.9 vs. 10.1 months) and progression-free survival (PFS; median, 25.9 vs. 7.6 months). In the retrospective study, 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. Both studies were limited by their small sample size. There is no direct evidence that the use of the test improves health outcomes. Due to the studies 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. There is insufficient evidence to determine that the technology results in an improvement in net health outcomes.

For individuals with Merkel cell carcinoma who receive tumor-informed ctDNA testing with Signatera to guide treatment decisions and monitor for recurrence, the evidence includes 2 prospective observational studies from a single multicenter cohort. Relevant outcomes are overall survival, disease-specific survival, test accuracy and validity, morbid events, and medication use. The available evidence supports an association between ctDNA status and recurrence risk, with high sensitivity and specificity for detecting clinically evident disease at enrollment, strong prognostic separation between ctDNA-positive and ctDNA-negative individuals during surveillance, and a median lead time of 2.7 months between the first positive ctDNA test and clinical recurrence. However, study limitations preclude drawing definitive conclusions about clinical validity, including an observational design without comparison to standard surveillance methods, enrollment limited to tertiary care centers, real-world variations in imaging and ctDNA collection intervals across sites, and median follow-up shorter than the 2- to 5-year window during which most Merkel cell carcinoma recurrences occur. No study reported management changes made in response to ctDNA test results, and no evidence addresses clinical utility. There is insufficient evidence to determine that the technology results in an improvement in net health outcomes.

For individuals with solid tumors who receive tumor-informed ctDNA testing with Signatera to monitor response to immunotherapy, the evidence includes a systematic review evaluating ctDNA as a predictive biomarker of pathologic complete response in patients with solid tumors who are treated with neoadjuvant immune checkpoint inhibitor (ICI) therapy and 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. In the systematic review, the estimated sensitivity of ctDNA clearance for pathologic complete response was 0.98 (95% confidence interval [CI], 0.86 to 1.00), and the estimated specificity of ctDNA persistence for lack of pathologic complete response was 0.53 (95% CI, 0.37 to 0.69). 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% CI, 0.29 to 0.83) and PFS (adjusted HR, 0.54; 95% CI, 0.34 to 0.85). The systematic review was limited by heterogeneity in the patient population and lack of a comparison to standard methods of monitoring response to treatment, whereas the study was also limited by a lack of comparison to standard methods of monitoring, in addition to a small sample size and variability in results across different tumor types. 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. There is insufficient evidence to determine that the technology results in an improvement in net health outcomes.

Rationale

Evidence reviews assess clinical evidence to determine whether the use of a technology improves the net health outcome. Broadly defined, health outcomes are length of life, quality of life, and ability to function including benefits and harms. Every clinical condition has specific outcomes that are important to individuals and to managing the course of that condition. Validated outcome measures are necessary to ascertain whether a condition improves or worsens; and whether the magnitude of that change is clinically significant. The net health outcome is a balance of benefits and harms. The following is a summary of the key literature to date.

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.

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). Two additional systematic reviews by international author groups had similar findings.^{3,4} A third systematic review and meta-analysis conducted by Camblor et al (2026) to assess whether tumor-informed (i.e., Signatera) or tumor-agnostic ctDNA assays had better testing performances while simultaneously considering the impact of the sampling strategy (landmark vs. serial).⁵ In the serial-sampling setting, tumor-informed (i.e., Signatera) assays demonstrated markedly higher sensitivity than tumor-agnostic assays (0.88 vs. 0.59; $p=.001$), while analyses of landmark sampling did not show a statistically significant difference, highlighting the importance of a serial sampling strategy.

Exploratory and/or Post Hoc Analyses from Randomized Trials

Three biomarker analyses from randomized trials evaluated Signatera ctDNA testing in colorectal cancer. Taieb et al (2025) assessed ctDNA and Immunoscore in 554 individuals with stage III colon cancer from the phase III IDEA-France and HORG-IDEA-Greece trials (median follow-up, 6.7 years).⁶ Zhang et al (2026) assessed ctDNA in 940 individuals with stage III colon cancer from the phase III CALGB/SWOG 80702 trial (median follow-up, 6.0 years).⁷ Akiyoshi et al (2025) assessed longitudinal ctDNA in 64 individuals with locally

advanced rectal cancer from the phase II NOMINATE trial (median follow-up, 16 to 18 months).⁸ Across all 3 studies, postoperative ctDNA positivity was consistently and strongly prognostic, with adjusted hazard ratios ranging from 4.84 to 6.12 for overall survival or disease-free survival in the 2 large stage III trials, and an HR of 6.7 for disease-free survival in the rectal cancer cohort. ctDNA positivity rates were consistent at approximately 19% in stage III colon cancer trials. In the NOMINATE trial, ctDNA positivity at final restaging had 100% specificity and positive predictive value for pathologic residual disease. However, sensitivity for detecting residual disease was limited, as 55% of individuals with residual tumors at surgery were ctDNA-negative at restaging. Across the 2 larger trials, 19% of ctDNA-negative individuals in IDEA-France/IDEA-Greece experienced recurrence or death within 5 years, and Zhang et al reported an association between celecoxib and improved disease-free survival in ctDNA-positive individuals (adjusted HR: 0.61; 95% CI, 0.42 to 0.89), though the treatment-by-ctDNA interaction was not statistically significant in the full cohort ($p=.41$). Limitations include, but are not limited to, the post hoc or companion biomarker design of all 3 studies, substantial participant attrition in both large trials (554 of 2,717 in IDEA and 940 of 2,526 in CALGB/SWOG 80702), single-timepoint ctDNA assessment in the 2 stage III trials precluding evaluation of clearance dynamics, short follow-up and modest sample size in the NOMINATE trial, and the absence of prospective evaluation of ctDNA-guided management decisions in any of the 3 studies.

Nonrandomized Trials

Several nonrandomized studies, most 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. Four 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.⁹ 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.¹⁰ 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 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.¹¹

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.¹² 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).⁹ 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.¹³ 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. An updated analysis was published with data through June 2024.¹⁴ This analysis included patients with stage II to III colon cancer or stage IV CRC and had a median follow-up of 23 months. Similar to the previous analysis, ctDNA positivity at 4 weeks after surgery was associated with a significantly higher risk of recurrence (disease free survival [DFS] HR, 11.99; p<.0001) and identified patients with high-risk stage II or III CRC who derived a benefit from adjuvant chemotherapy (HR, 0.23; p<.0001).

Molinari et al (2024) analyzed post-neoadjuvant therapy and postsurgical ctDNA levels in a retrospective study of 33 patients with stage II to III rectal cancer.¹⁵ After a median follow-up of 60 months, post-neoadjuvant therapy ctDNA positivity was associated with a significantly higher risk of recurrence (HR, 7.82), as was ctDNA positivity at 2 to 12 weeks post-surgery (HR, 19.65). Estimates were imprecise, with wide confidence intervals.

Slater et al (2024) prospectively analyzed ctDNA levels in patients with resectable stage I to III CRC before and after surgery, post-adjuvant chemotherapy, every 3 months for year 1 and every 6 months in years 2 and 3.¹⁶ A total of 1203 patients were enrolled, of whom plasma samples were available for 214 patients; the median follow-up was 30.3 months. The 2-year RFS was 91.1% in patients with ctDNA not detected postoperatively and 50.4% in those with ctDNA detected (HR, 6.5; p<.0001). The negative predictive value, sensitivity, and specificity were 88.5%, 41.7%, and 90.8%, respectively. The TRACC Part C study is currently investigating the potential de-escalation of adjuvant chemotherapy in patients with undetectable postoperative ctDNA.

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) ¹⁰	1. Risk stratification 2. Monitoring response to	130 individuals with stages I to III CRC;	Multicenter, Denmark	CEA and CT imaging	2 or more variants detected out of 16	Before and after surgery, during and after adjuvant	Yes

	adjuvant chemotherapy 3. Early relapse detection	treated from May 1, 2014, to January 31, 2017				chemotherapy, and during surveillance Sample at Day 30 following surgery; individuals were followed up for a median of 12.5 months	
Henriksen et al (2022) ¹¹ ,	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 therapy; individuals were followed up for a median of 35 months.	Yes
Loupakis et al (2021) ¹² ,	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) ⁹ ,	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	No

						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 resected stage IV disease.	
Kotani et al (2023) ¹³ , Nakamura et al (2024) ¹⁴ ,	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 (Kotani et al [2023]) and updated analysis of 2,240 individuals with stage II to III resectable colon cancer or stage IV CRC through June 2024 (Nakamura et al [2024])	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
Molinari et al (2024) ¹⁵ ,	1. Risk stratification following neoadjuvant therapy and following surgery	33 individuals with stage II to III rectal cancer, diagnosed between 2017 and 2021	Italy	NR	ctDNA detected-greater or equal to 2 variants detected out of 16	Samples collected after diagnostic biopsy, before surgery, and 2 to 12 weeks after surgery	No

Slater et al (2024); TRACC Part B study ¹⁶ ,	1. Risk stratification 2. Monitoring response to adjuvant chemotherapy 3. Early relapse detection	1,203 individuals with stage I to III resectable CRC from 2016 to 2022; plasma samples from 212 individuals were analyzed	UK	Radiological imaging	NR	Samples collected before and 3 to 12 weeks after surgery, after adjuvant chemotherapy, and every 3 months for year 1 and every 6 months in years 2 and 3	NR
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CEA: carcinoembryonic antigen; CRC: colorectal cancer; CT: computerized tomography; ctDNA: circulating tumor DNA ; IQR: interquartile range; NCCN: National Comprehensive Cancer Network; NR: not reported; TRACC: TRacking mutations in cell-free tumor DNA to predict relapse in early Colorectal Cancer

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) ¹⁰ ,	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) ¹¹ ,	16/20 (80%)	22/120 (18%)
HR for RFS (95% CI)	7.0 (3.7 to 13.5); p<.001	
Loupakis et al (2021) ¹² ,	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) ¹³ ,	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	
Nakamura et al (2024) ¹⁴ ,	336/2109 (15.93%)	1773/2109 (84%)
HR for DFS (95% CI)	11.99 (10.02 to 14.35); p<.0001	
HR for overall survival (95% CI)	9.68 (6.33 to 14.82); p<.0001	
Patients with stage II to III colon cancer	192/1,349 (14.23%)	1,157/1,349 (85.77%)
HR for benefit with adjuvant chemotherapy (95% CI)	0.23 (0.15 to 0.35); p<.0001	
Molinari et al (2024) ¹⁵ ,	At baseline, ctDNA detection rate was 90% (27/30) post-neoadjuvant therapy, ctDNA was detected in 5 patients post-surgery, ctDNA was detected in 3 patients	
HR for DFS post-neoadjuvant therapy (95% CI)	7.82 (2.28 to 26.82); p<.001	
HR for DFS post-surgery (95% CI)	19.65 (3.17 to 121.75); p<.001	
Slater et al (2024) ¹⁶ ,	21/143 (14.7%)	122/143 (85.3%)

HR for RFS (95% CI)	6.5 (3 to 14.5); p<.0001
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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) ⁹ .					
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
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 Signatera vs. imaging Signatera vs. imaging plus CEA Signatera vs. CEA	>.99 .55 .13	>.99 .13 .25	NA	NA	.45 .79 NA

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) ¹⁰ .	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) ¹¹ .			3. No comparator		1. Follow up for recurrence was under 3 years (median 35 months)
Loupakis et al (2021) ¹² .			3. No comparator		1. Follow up for recurrence was under 3 years (median 10.7 months)
Fakih et al (2022) ⁹ .				1. Survival outcomes not assessed	
Kotani et al (2023) ¹³ ;			3. No comparator	1. Overall survival not assessed	1. Follow up for recurrence was under 3

Nakamura et al (2024) ¹⁴ ,					years (median 16.74 months)
Molinari et al (2024) ¹⁵ ,			3. No comparator	1. Overall survival not assessed	
Slater et al (2024) ¹⁶ ,	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 30.3 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) ¹⁰ ,	1. individual selection not described					Multiple subgroup analyses, small numbers of individuals with positive ctDNA tests.
Henriksen et al (2022) ¹¹ ,			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) ¹² ,						Small numbers of individuals with positive ctDNA tests.
Fakih et al (2022) ⁹ ,						
Kotani et al (2023) ¹³ , Nakamura et al (2024) ¹⁴ ,	1. individual selection not described					
Molinari et al (2024) ¹⁵ ,	1. individual selection not described					

Slater et al (2024) ¹⁶ .	1. individual selection not described	1. blinding not described				
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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, multiple noncomparative studies (N = 2895), and 1 retrospective comparative study (N = 48). The systematic reviews and nonrandomized studies have reported an association between ctDNA results and prognosis when measured at diagnosis, following neoadjuvant therapy and surgery, during adjuvant therapy, and during surveillance after curative treatment , 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.

Systematic Reviews

Zhang et al (2025) published a systematic review investigating the prognostic value of ctDNA in early-stage triple-negative breast cancer (TNBC).¹⁷ Twenty publications were included in the analysis, though only 4 studies specifically investigated the Signatera assay. Results demonstrated that post-neoadjuvant (before or after surgery) ctDNA positivity was associated with increased risk of disease recurrence (HR, 4.12, 95% CI, 2.81 to 6.04; p<.00001) as well as worse overall survival (HR, 3.26; 95% CI, 1.88 to 5.63; p<.0001).

Ali et al (2026) conducted a systematic review and meta-analysis of 19 studies (only 2 of the studies specifically used the Signatera assay) to evaluate the prognostic value of ctDNA for predicting early recurrence and survival outcomes in early-stage breast cancer (stage I–III) across all molecular subtypes.¹⁸ For recurrence-free survival endpoints (disease-free survival [DFS], relapse-free survival [RFS], or event-free survival [EFS]), ctDNA positivity was associated with increased risk of recurrence or poor survival outcomes (pooled HR: 3.28; 95% CI, 1.81 to 5.93), though substantial heterogeneity was observed (I²=93.3%). Pooled estimates

for overall survival (HR: 8.92; 95% CI, 0.45 to 177.87; 3 studies) and recurrence/relapse (HR: 5.21; 95% CI, 0.98 to 27.69) were imprecise, with confidence intervals crossing the null, and were also plagued with substantial heterogeneity ($I^2=74.4\%$ and 84.4% , respectively). Sensitivity analysis excluding studies with high risk of bias yielded a pooled HR of 2.47 (95% CI, 1.89 to 3.23; $I^2 = 0\%$). Significant publication bias was detected (Egger's $p=.018$). A subgroup analysis based on assay types indicated that customized ctDNA assays yielded more consistent and lower heterogeneity estimates (HR: 0.81 [0.10; 6.86], $I^2= 0\%$) when compared to tumor-informed assays (HR: 2.66, 0.48 to 14.84], $I^2=87.9\%$), suggesting these assays are more reliable. The authors concluded that ctDNA positivity was strongly associated with recurrence risk but noted that prospective trials are needed to establish clinical utility, and that none of the included studies prospectively tested ctDNA-guided treatment modifications as a primary objective.

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.¹⁹ 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.²⁰ 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) ¹⁹ .	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) ²⁰	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
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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) ¹⁹	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) ²⁰	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
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Coombes et al (2019) ¹⁹ .	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) ²⁰ .			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) ¹⁹ .						1. Confidence intervals for test characteristics not reported; small number of positive ctDNA tests
Magbanua et al (2021) ²⁰ .	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 systematic reviews and 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. The systematic review included patients with early-stage TNBC and found that post-neoadjuvant (before or after surgery) ctDNA positivity was

associated with a worse prognosis. 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.

Randomized Controlled Trial

Powles et al (2025) reported results from IMvigor011, a phase 3, double-blind, randomized controlled trial (RCT) evaluating ctDNA-guided adjuvant atezolizumab versus placebo in patients with muscle-invasive bladder cancer after cystectomy.²¹ A total of 761 individuals underwent serial ctDNA monitoring with Signatera (or a comparable BGI assay in mainland China) for up to 1 year after surgery with 250 patients who tested ctDNA-positive randomized 2:1 to atezolizumab (n=167) or placebo (n=83). Among ctDNA-positive individuals, those randomized to atezolizumab had statistically significantly longer disease-free survival and overall survival compared with placebo. Among 357 patients with persistent ctDNA-negative status, the majority remained disease-free through the surveillance period. Despite these promising results, no definitive conclusions can be drawn about the clinical utility of the ctDNA Signatera test since the RCT was evaluating outcomes based on adjuvant use of atezolizumab, which is no longer FDA-approved for urothelial carcinoma following the withdrawal of its accelerated approval after the IMvigor010 failed to meet its primary endpoint. The authors concluded that among patients with muscle-invasive bladder cancer, ctDNA-guided adjuvant therapy with atezolizumab led to significantly longer disease-free survival and overall survival than placebo.

Nonrandomized Trials

Several 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).²² Data from a 68-month follow-up of this cohort were reported by Linskrog et al (2023).²³ Additionally, Linskrog 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. Study results suggest that ctDNA analysis is better associated with treatment efficacy compared with other available methods.

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.²⁴ 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.²⁵

Lindskrog et al (2023) presented full follow-up results (median follow-up: 68 months) from a previously described cohort of 68 neoadjuvant chemotherapy (NAC) treated patients who underwent longitudinal ctDNA testing (712 plasma samples). In addition, we performed ctDNA evaluation of 153 plasma samples collected before and after radical cystectomy (RC) in a separate cohort of 102 NAC-naïve patients (median follow-up: 72 months). Total RNA sequencing of tumors was performed to investigate biological characteristics of ctDNA shedding tumors. Assessment of ctDNA after RC identified metastatic relapse with a sensitivity of 94% and specificity of 98% using the expanded follow-up data for the NAC-treated patients. ctDNA dynamics during NAC was independently associated with patient outcomes when adjusted for pathologic downstaging (HR = 4.7; $P = 0.029$). For the NAC-naïve patients, ctDNA was a prognostic predictor before (HR = 3.4; $P = 0.0005$) and after RC (HR = 17.8; $P = 0.0002$). No statistically significant difference in recurrence-free survival for patients without detectable ctDNA at diagnosis was observed between the cohorts. Baseline ctDNA positivity was associated with the Basal/Squamous (Ba/Sq) subtype and enrichment of epithelial-to-mesenchymal transition and cell cycle-associated gene sets. ctDNA is prognostic in NAC-treated and NAC-naïve patients with more than 5 years follow-up and outperforms pathologic downstaging in predicting treatment efficacy.

Ben-David et al (2024) retrospectively assessed whether ctDNA status before cystectomy and during surveillance is predictive of recurrence in patients with bladder cancer.²⁶ A total of 112 patients were included with a median follow-up of 8 months. Detectable ctDNA before surgery was found in 59 patients and was associated with worse prognosis (Kaplan-Meier analysis, $p<.0001$). Additionally, patients with undetectable ctDNA before surgery had comparable RFS regardless of whether they received neoadjuvant therapy before surgery. Patients with detectable ctDNA that remained persistently detectable after surgery had the worst prognosis, with RFS rates of 33% at 6 months and 16% at 12 months. The authors concluded that detectable ctDNA before definitive therapy with radical cystectomy is predictive of nodal involvement, locally advanced disease, and disease recurrence in patients with bladder cancer.

Sfakianos et al (2025) retrospectively assessed whether ctDNA status before cystectomy and during surveillance is predictive of recurrence in patients with bladder cancer.²⁷ A total of 167 patients were included with a median follow-up of 10 months. Detectable ctDNA before surgery was found in 26 of 65 patients (40%) with available plasma samples. Among those receiving upfront surgery, patients with detectable ctDNA had shorter DFS (HR, 10.61; 95% CI, 1.01 to 1434; $p=.03$). Likewise, detectable ctDNA during the window of 1 to 12 weeks after surgery was associated with shorter DFS (HR, 6.93; $p<.001$) as well as during surveillance (more than 12 weeks after surgery if no adjuvant therapy was administered or 2 or more weeks after the completion of adjuvant therapy; HR, 23.02; $p<.001$). The authors concluded that the ctDNA

testing in patients with bladder cancer undergoing radical cystectomy was prognostic and potentially predictive. Identification of patients at high risk of recurrence can aid in patient counseling and decision-making.

Galsky et al (2025) reported 5-year results from the CheckMate 274 trial, a phase III, double-blind, randomized trial comparing adjuvant nivolumab versus placebo in 709 individuals with high-risk muscle-invasive urothelial carcinoma (MIUC) after radical surgery.²⁸ In a post hoc exploratory analysis, baseline ctDNA was measured using the Signatera assay in 133 assessable individuals with 54 (40.6%) having detectable ctDNA. Individuals with detectable ctDNA had substantially shorter disease-free survival than those with undetectable ctDNA (median, 5.0 vs. 52.1 months; HR: 0.30; 95% CI, 0.18 to 0.48). Among those with detectable ctDNA, nivolumab was associated with longer disease-free survival and overall survival compared with placebo, whereas no such difference was observed among patients with undetectable ctDNA suggesting that monitoring ctDNA may be prognostic of recurrence. Notable limitations include, but are not limited to, the post-hoc and exploratory nature of the ctDNA analysis, the small sample size of the subgroup (133 of 709 randomized, 18.8%), absence of serial ctDNA monitoring (single landmark timepoint only), and the lack of prospective ctDNA-guided treatment assignment. Five-year results confirm durable disease-free survival benefit and consistent safety profile for adjuvant nivolumab in high-risk MIUC. Post hoc exploratory ctDNA assessment identified patients at highest risk of recurrence and may inform tailored adjuvant therapy strategies, pending further validation.

Gao et al (2025) conducted a systematic review and meta-analysis evaluating whether plasma circulating tumor DNA (ctDNA) status has prognostic and predictive value in patients with muscle-invasive bladder cancer (MIBC). The authors searched PubMed, EMBASE, and the Cochrane Library through December 2023 and included 11 studies comprising 1,170 patients with MIBC; the evidence base included four retrospective cohort studies and eight prospective cohort studies, with one study contributing two cohorts. Overall, ctDNA positivity was strongly associated with worse survival outcomes. Patients with detectable ctDNA had significantly poorer overall survival (HR 4.51; 95% CI, 2.64–7.69), progression-free survival (HR 4.50; 95% CI, 2.77–7.30), and recurrence-free survival (HR 6.56; 95% CI, 4.18–10.30), with prognostic significance observed both before and after treatment. Longitudinal ctDNA monitoring also appeared clinically informative: ctDNA clearance was associated with improved recurrence-free survival (HR 0.24; 95% CI, 0.14–0.41). The authors concluded that ctDNA status is a promising prognostic and predictive biomarker in MIBC and may be useful for monitoring recurrence and guiding treatment decisions.

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
Galsky et al (2025) ²⁸ ,						
Sfakianos et al (2025) ²⁷ ,	167 individuals with stage I to IV bladder cancer	Retrospective, multicenter in the US	Radiological imaging	Greater or equal to 2 variants	Blood samples collected before and after	NR

	who underwent cystectomy between 2016 and 2023			detected out of 16	cystectomy. Medan follow-up of 10 months	
Ben-David et al (2024) ²⁶ ,	112 individuals with bladder cancer who underwent cystectomy between 2021 and 2023	Retrospective, 1 hospital in the US	Radiological imaging	NR	Blood samples collected after completion of neoadjuvant therapy (if applicable) and before and after cystectomy. Median follow-up of 8 months.	No
Lindskrog et al (2023) ²³ ,	102 individuals with muscle-invasive bladder cancer who underwent cystectomy between 2001 and 2014 and did not receive neoadjuvant chemotherapy	Retrospective, 1 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
Powles et al (2021) ²⁴ ,	581 individuals with urothelial cancer from a randomized Phase III trial of adjuvant atezolizumab vs. observation who had undergone surgery and were evaluable for ctDNA	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 weeks after randomization and individuals were followed for a median of 23 months	No
Christensen et al (2019) ^{22,23} ,	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]).	Yes

					Median follow-up of 68 months after cystectomy (published by Lindskrog et al [2023]).	
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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
Sfakianos et al (2025) ²⁷ ,		
Before cystectomy	26/65 (40%)	39/65 (60%)
DFS	Median, 7.98 months	Not reached
HR for DFS (95% CI)	10.61 (1.01 to 1434) p=.035	
1 to 12 weeks after cystectomy	24/100 (24%)	76/100 (76%)
Recurrence	8/18 (44%)	6/76 (8%)
HR for DFS (95% CI)	HR, 6.93 (2.4 to 20.05); p<.001	
12 weeks after cystectomy if no adjuvant therapy or 2 or more weeks after the completion of adjuvant therapy	52/117 (44%)	65/117 (56%)
DFS	Median, 14.1 months	Not reached
HR for DFS (95% CI)	23.02 (5.51 to 96.17); p<.001	
Ben-David et al (2024) ²⁶ ,		
Before cystectomy	59/112 (53%)	53/112 (47%)
RFS at 6 months	64%	98%
RFS at 12 months	35%	95%
RFS at 15 months	35%	76%
Lindskrog et al (2023) ²³ ,		
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) ^{22,23} ,		
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) ²⁴ .		
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; RFS: recurrence-free survival.

Table 12. Study Relevance Limitations

Study	Population ^a	Intervention ^b	Comparator ^c	Outcomes ^d	Duration of Follow-Up ^e
Sfakianos et al (2025) ²⁷ .			3. Not compared to tests used for the same purpose		Short follow-up of 10 months
Ben-David et al (2024) ²⁶ .			3. Not compared to tests used for the same purpose		Short follow-up of 8 months
Lindskrog et al (2023) ²³ .			3. Not compared to tests used for the same purpose		
Powles et al (2021) ²⁴ .			3. Not compared to tests used for the same purpose		
Christensen et al (2019) ²² .			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
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Sfakianos et al (2025) ²⁷ ,						
Ben-David et al (2024) ²⁶ ,						
Lindskrog et al (2023) ²³ ,		Blinding not described.				
Powles et al (2021) ²⁴ ,						
Christensen et al (2019) ²² ,						1. Confidence intervals for hazard ratios 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 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 an RCT, 1 uncontrolled prospective cohort study (N = 68), 3 retrospective cohort studies (N = 381), and 2 retrospective subgroup analyses from RCTs (N = 581). The RCT demonstrated an association between test results and response to atezolizumab treatment. The prospective study reported an association between Signatera test results at diagnosis, during chemotherapy treatment, and during surveillance following cystectomy to prognosis. The retrospective studies reported an association between Signatera test results at diagnosis and during surveillance following cystectomy to prognosis; patients received chemotherapy in 2 of these studies. The retrospective subgroup analysis reported an association between test results and response to atezolizumab treatment.

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 courses to inform treatment decisions and to monitor for recurrence following surgical resection.

Systematic Reviews

Lu et al (2025) published a systematic review and meta-analysis evaluating the diagnostic performance of ctDNA-based minimal residual disease (MRD) testing for recurrence detection in postoperative early-stage NSCLC.²⁹ Thirty studies comprising of 3,287 individuals were included, using various detection strategies (17 tumor-agnostic, 12 tumor-informed, 1 tumor-naïve), with 5 of the 30 studies specifically using the Signatera assay. At the landmark timepoint (single assessment within 3 months of surgery), pooled sensitivity was 0.44 (95% CI, 0.38 to 0.50), specificity was 0.95 (95% CI, 0.93 to 0.97), and area under the curve (AUC) was

0.73 (95% CI, 0.69 to 0.77). Longitudinal monitoring (serial assessments) improved sensitivity to 0.78 (95% CI, 0.70 to 0.85) with specificity of 0.92 (95% CI, 0.85 to 0.96) and AUC of 0.91 (95% CI, 0.88 to 0.93). In subgroup analysis, tumor-informed assays demonstrated higher specificity at landmark (0.97 vs. 0.93) and AUC (0.81 vs. 0.70) than tumor-agnostic approaches, while differences narrowed in longitudinal monitoring. No evidence of publication bias was detected. The authors concluded that ctDNA-based MRD testing provides clinically meaningful prognostic information, though standardization of detection methods and clear guidelines for clinical use remain needed. Notable limitations include heterogeneity among included studies, majority of the studies were conducted in Asia, and incomplete reporting of clinical variables across included studies.

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 and a single-center pilot study of 25 individuals with stage IA to IIIA EGFR-mutant NSCLC.^{30,31} 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%). Lee et al (2025) reported exploratory ctDNA findings that indicated 6 individuals (30%) were ctDNA-positive at baseline and achieved clearance after 1 cycle of neoadjuvant osimertinib, however, the study failed its primary endpoint, ctDNA analysis was exploratory, and the small sample size and single recurrence event preclude conclusions about the prognostic or predictive value of Signatera testing in this population.

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) ³⁰	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).
Lee et al (2025) ³¹ ,	25 individuals with stage IA to IIIA EGFR-mutant NSCLC	Single-center, open-label pilot study; exploratory ctDNA endpoint	Clinical assessment and chest radiograph	2 or more SNVs detected above predefined confidence threshold	Before neoadjuvant osimertinib, cycle 2 day 1, day of surgery, and every 2 to 3 months during adjuvant treatment	Not described	6 of 20 patients (30%) were ctDNA-positive at baseline; all achieved clearance after 1 cycle. 18 of 19 recurrence-free patients were ctDNA-negative (95%). One recurrence event at median follow-up of 31 months

CT: computed tomography; ctDNA: circulating tumor DNA; NSCLC: non-small cell lung cancer; SNV: single nucleotide variant.

Table 15. Study Relevance Limitations

Study	Population ^a	Intervention ^b	Comparator ^c	Outcomes ^d	Duration of Follow-Up ^e
Abbosh et al (2017) ³⁰ ,			3. No comparison to standard methods of monitoring for relapse	1. Health outcomes not assessed	
Lee et al (2025) ³¹ ,			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) ³⁰ ,	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

Lee et al (2025) ³¹ ,		1. Open label; not blinded to treatment or results			2. 5 of 25 patients excluded from ctDNA analysis	1. No comparison to imaging, no confidence intervals
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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 a systematic review, 2 subgroup analyses of participants enrolled in prospective observational studies. The systematic review demonstrated in a subgroup analysis that tumor-informed assays demonstrated higher specificity at landmark (0.97 vs. 0.93) and AUC (0.81 vs. 0.70) than tumor-agnostic approaches, while differences narrowed in longitudinal monitoring. Of 14 individuals with confirmed relapses, 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.

Systematic Review

Wang et al (2025) published a systematic review and meta-analysis to assess the prognostic value of ctDNA in individuals with esophageal cancer (EC).³² Twenty-two studies involving 1,519 individuals were included, however, none of the included studies used the Signatera assay. In multivariate analyses, ctDNA positivity was associated with poorer progression-free survival (PFS) at baseline (HR:2.88, 95% CI:1.95 to 4.25), after neoadjuvant therapy (HR: 4.21, 95% CI:2.67 to 6.64), and during follow-up (HR:4.11, 95% CI:1.86 to 9.08). Similarly, ctDNA positivity was associated with poorer overall survival (OS) at all timepoints (baseline: HR:3.79, 95% CI:1.48 to 9.65; after neoadjuvant therapy: HR:2.70, 95% CI:1.34 to 5.41; follow-up: HR:6.69, 95% CI:3.54 to 12.62), with combined univariate and multivariate HRs increasing progressively from baseline to follow-up. Furthermore, ctDNA positivity predicted clinical recurrence an average of 4.53 months earlier (range, 0.98 to 11.6 months) than conventional imaging. The authors concluded that ctDNA's prognostic value increased over

time from baseline to follow-up, supporting its potential as a dynamic monitoring biomarker, though standardized detection thresholds and prospective intervention trials remain needed. Notable limitations include, but not limited to, bias and heterogeneity within clinical and methodological variables across studies, lack of detailed clinical information regarding cancer subtypes, and inconsistent outcome measures.

Nonrandomized Trials

One prospective cohort study and 1 noncomparative retrospective study reported the association of Signatera testing measured before and after surgery with relapse and recurrence (Tables 17 and 18).

Mukherjee et al (2025) examined the association between ctDNA and prognosis in a Phase II study of 22 patients with localized esophageal or gastroesophageal junction adenocarcinoma treated with induction chemotherapy and preoperative chemoradiation.³³ With a median follow-up of 15.8 months, ctDNA data were available for 10 patients, and 7 were eligible for survival analysis. Among them, patients who cleared ctDNA post-surgery (n=5) had significantly better overall survival (25.9 vs. 10.1 months; p=.012) and progression-free survival (PFS; 25.9 vs. 7.6 months; p=.0082) than those with persistent ctDNA (n=2).

Ococks et al (2021) assessed data from 17 individuals with esophageal adenocarcinoma. 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
Mukherjee et al (2025) ³³ ,	22 individuals with localized esophageal or gastroesophageal junction adenocarcinoma treated with induction chemotherapy and preoperative chemoradiation followed by surgery	Prospective	Radiological imaging	2 or more variants detected out of 16	Blood samples were collected at baseline, after induction chemotherapy, after chemoradiation, and after surgery, and patients were followed up for a median of 15.8 months	No
Ococks et al (2021) ³⁴ ,	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			
	Overall survival	PFS	
Mukherjee et al (2025) ³³ ,			
ctDNA status following surgery	ctDNA positive: n=2 ctDNA negative: n=5	ctDNA positive: n=2 ctDNA negative: n=5	
ctDNA clearance vs no clearance following surgery	median, 25.9 vs. 10.1 months; p=.012	median, 25.9 vs. 7.6 months; p=.0082	
	Median DFS		
	ctDNA Positive	ctDNA Negative	p for comparison
Ococks et al (2021) ³⁴ ,			
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 survival; NR: not reported; PFS: progression-free survival.

Table 19. Study Relevance Limitations

Study	Population ^a	Intervention ^b	Comparator ^c	Outcomes ^d	Duration of Follow-Up ^e
Mukherjee et al (2025) ³³ ,	4. The study included 54.5% of patients with gastroesophageal junction adenocarcinoma and 45.5% of patients with esophageal adenocarcinoma		3. No comparison to tests used for the same purpose		1. Short follow-up of approximately 16 months
Ococks et al (2021) ³⁴ ,		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
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Mukherjee et al (2025) ³³						Imprecise estimates due to small sample size
Ococks et al (2021) ³⁴					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 a systematic review, a noncomparative prospective study (N = 22) and 1 noncomparative, retrospective study (N = 17). The systematic review demonstrated that in a multivariate analysis, ctDNA positivity was associated with poorer progression-free survival (PFS) at baseline, after neoadjuvant therapy, and during follow-up. In the prospective study, clearance of ctDNA post-surgery was associated with improved overall survival (median, 25.9 vs. 10.1 months) and PFS (median, 25.9 vs. 7.6 months). In the retrospective study, 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. Both studies were limited by their small sample size. There is no direct evidence that the use of the test improves health outcomes. Due to the studies 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 Merkel Cell Carcinoma

Clinical Context and Test Purpose

The purpose of Signatera testing in individuals who have Merkel cell carcinoma (MCC) is to inform treatment decisions and to monitor for recurrence following curative treatment.

Nonrandomized Trials

Akaike et al (2024) conducted a prospective, multicenter, observational study evaluating Signatera ctDNA testing in 319 individuals with stage I–IV Merkel cell carcinoma (MCC) enrolled across 6 U.S. tertiary care centers, with a discovery cohort (n=167) and prespecified validation cohort (n=152).³⁵ At enrollment, ctDNA sensitivity for clinically evident disease was 95% (discovery) and 94% (validation), with corresponding specificities of 90% and 86%. During surveillance, ctDNA positivity at any timepoint was strongly associated with subsequent recurrence (HR: 6.8; 95% CI, 2.9 to 16; $p < .001$ in the discovery cohort; HR: 20; 95% CI, 8.3 to 50; $p < .001$ in the validation cohort). The negative predictive value was strongest at 135 days after a negative test and was 94% and 93% for the discovery and validation cohorts, respectively, with higher levels of ctDNA significantly associated with an increased probability of clinical recurrence. In a subset of individuals with stage I–III MCC (n= 84) who underwent

ctDNA testing within 4 months of curative-intent treatment, ctDNA positivity was associated with a significantly higher 1-year recurrence rate than ctDNA negativity (74% vs. 21%; adjusted HR: 7.4; 95% CI, 2.7 to 20). Limitations include, but are not limited to, the observational design without comparison to standard surveillance methods, enrollment limited to tertiary care centers, heterogeneity in treatment modalities, imaging, and ctDNA collection intervals across the 6 centers, and insufficient follow-up (median of 295 days).

Akaike et al (2026) reported a secondary analysis of 215 individuals from the same prospective observational cohort, evaluating the temporal relationship between the first positive Signatera ctDNA test and subsequent clinical recurrence across 669 ctDNA tests during surveillance.³⁶ Individuals with at least one positive ctDNA test during surveillance were more likely to experience recurrence than persistently ctDNA-negative individuals (adjusted HR: 18.1; 95% CI, 8.9 to 36.7), with 77% developing clinically evident disease within 1 year of the first positive test. The 29 recurrences preceded by ctDNA positivity, the median lead time from first positive ctDNA to clinical recurrence was 2.7 months (95% CI, 1.9 to 4.7). Quantitative ctDNA levels at first positive test were associated with time to recurrence, with median times of 7.5 months, 2.8 months, and 1.3 months for levels below 1 mean tumor molecule per mL (MTM/mL), 1 to 10 MTM/mL, and above 10 MTM/mL, respectively. Among initially ctDNA-negative individuals who remained negative, the risk of clinical recurrence at 3, 6, and 12 months of surveillance was 0.6%, 4.6%, and 7.7%, respectively. Limitations include, but are not limited to, the secondary analytical nature of the study with the same cohort and limitations as Akaike et al (2024), heterogeneity in stage, prior treatment type and timing, and history of recurrence, and absence of evidence that ctDNA-guided management decisions alter clinical outcomes.

Section Summary: Tumor-Informed Circulating Tumor DNA Testing with Signatera in Individuals with Merkel Cell Carcinoma

For individuals with Merkel cell carcinoma who receive tumor-informed ctDNA testing with Signatera to guide treatment decisions and monitor for recurrence, the evidence includes 2 prospective observational studies from a single multicenter cohort (N = 319 in the primary study; N = 215 in a secondary analysis). The primary study demonstrated high sensitivity (94% to 95%) and specificity (86% to 90%) for detecting clinically evident disease at enrollment, and serial ctDNA testing during surveillance was strongly associated with subsequent recurrence (HR ranging from 6.8 to 20 across discovery and validation cohorts), with post-treatment ctDNA positivity within 4 months of curative-intent treatment associated with a significantly higher 1-year recurrence rate compared with ctDNA negativity (74% vs. 21%; adjusted HR, 7.4). The secondary analysis reported a median lead time of 2.7 months between the first positive ctDNA test and clinical recurrence, with quantitative ctDNA levels associated with time to recurrence. The available evidence supports an association between ctDNA status and recurrence risk, however, study limitations preclude drawing definitive conclusions about clinical validity, including an observational design without comparison to standard surveillance methods, enrollment limited to tertiary care centers, real-world variations in imaging and ctDNA collection intervals across sites, and median follow-up shorter than the 2- to 5-year window during which most MCC recurrences occur. No study reported management changes made in response to ctDNA test results, and no evidence addresses clinical utility.

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.

Systematic Review

Valenza et al (2025) published a systematic review evaluating ctDNA as a predictive biomarker of pathologic complete response in patients with solid tumors who are treated with neoadjuvant immune checkpoint inhibitor (ICI) therapy.³⁷ Thirteen trials (N = 380) were included, of which only 2 trials specifically evaluated Signatera. Among the 380 patients, 74 received ICI monotherapy and 306 received an ICI in combination with chemotherapy or targeted therapy. Overall, 144 of 380 (38%) patients achieved pathologic complete response after neoadjuvant treatment, and 262 of 380 (73%) had ctDNA clearance after neoadjuvant ICI therapy. The estimated sensitivity of ctDNA clearance for pathologic complete response was 0.98 (95% CI, 0.86 to 1.00), and the estimated specificity of ctDNA persistence for lack of pathologic complete response was 0.53 (95% CI, 0.37 to 0.69).

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).³⁸

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). 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) ³⁸ ,	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

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

	Overall Survival
Bratman et al (2020) ³⁸ .	
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) ³⁸ .	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) ³⁸ .						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 systematic review evaluating ctDNA as a predictive biomarker of pathologic complete response in patients with solid tumors who are treated with neoadjuvant ICI therapy and a subgroup analysis of individuals enrolled in

a nonrandomized trial of pembrolizumab (N = 106). In the systematic review, the estimated sensitivity of ctDNA clearance for pathologic complete response was 0.98 (95% CI, 0.86 to 1.00), and the estimated specificity of ctDNA persistence for lack of pathologic complete response was 0.53 (95% CI, 0.37 to 0.69). 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

CLINICAL INPUT FROM PHYSICIAN SPECIALTY SOCIETIES, ACADEMIC MEDICAL CENTERS AND EXTERNAL STAKEHOLDERS (PRACTICING CLINICIANS AND RESEARCH BASED EXPERTS IN THE FIELD)

PRACTICE GUIDELINES AND POSITION STATEMENTS

Guidelines or position statements will be considered for inclusion in 'Supplemental Information' if they are 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 is 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 is 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)."

The American Society of Clinical Oncology (ASCO) 2022 guideline update on biomarkers for adjuvant endocrine and chemotherapy in early-stage breast cancer does not recommend the use of circulating tumor DNA (ctDNA) as a biomarker to guide decisions for adjuvant endocrine and chemotherapy (Type: evidence-based; Evidence quality: intermediate; Strength of recommendation: strong).³⁹ The guideline explicitly states the following recommendation:

- "If a patient has node-negative or node-positive ER-positive, HER2-positive, or TNBC, the clinician should not use ctDNA test to guide decisions for adjuvant endocrine and chemotherapy."

American Urological Association

In 2017, the American Urological Association published guidelines on treatment of non-metastatic muscle-invasive bladder cancer, which were amended in 2020 and 2024.⁴⁰ The guideline discusses circulating tumor DNA in regard to surveillance of individuals with non-metastatic muscle-invasive bladder cancer and states "Some evidence suggests a potential role for circulating tumor cell DNA (ctDNA) in detecting recurrence and progression following radical cystectomy. Future studies are needed to further define the potential role of ctDNA for surveillance."

National Comprehensive Cancer Network

The guidelines on colon and rectal cancer state: "Circulating tumor (ctDNA) is a prognostic marker; however, there is currently insufficient evidence to recommend routine use of ctDNA assays outside of a clinical trial. De-escalation of care and treatment decision-making are not recommended based on ctDNA results. Participation in clinical trials is encouraged. ctDNA is not recommended for surveillance."^{41,42}

The guideline on breast cancer makes several statements regarding ctDNA assays as noted below. The relevant discussion for these recommendations is pending an update.⁴³

- 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 for some alterations, but ctDNA may reflect tumor heterogeneity more accurately. If one specimen is negative for actionable biomarkers, testing on the alternative specimen can be considered."
- In HR-positive/HER2-negative breast cancer: "Some plasma-based ctDNA assays include a measure of tumor fraction (TF), which can aid in identification of low ctDNA content. Samples with low TF, especially <1%, should be interpreted with caution. Tests have varying sensitivities at low TF. Additional sampling from current tumor sample or future plasma can be considered."
- In HR-positive/HER2-negative breast cancer with ESR1 mutation: "Assess for ESR1 mutations at progression following prior lines of endocrine therapy (ctDNA preferred)."
- In recurrent unresectable (local or regional) or stage IV breast cancer: "Tissue biopsy is more sensitive than ctDNA (liquid biopsy) at detecting homozygous copy loss of PTEN or TMB-H."

The guideline on bladder cancer states the following regarding muscle-invasive bladder cancer/resectable: "The Panel recognizes that personalized, tumor-informed circulating tumor DNA (ctDNA) has emerged as a prognostic and predictive biomarker after cystectomy. The Panel currently recommends the consideration of ctDNA-MRD testing as a tool for risk stratification and to determine the use of adjuvant immunotherapy after cystectomy in patients who have not received previous immune checkpoint inhibitor treatment using an FDA-approved, personalized, tumor-informed, multiplex PCR-NGS assay for ctDNA."⁴⁴

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. The detection of mutations/alterations or fusions in DNA shed from esophageal or EGJ carcinomas can identify targetable alterations or the evolution of clones with altered treatment response profiles. Therefore, when limited tissue is available or for patients who have metastatic or advanced esophageal/esophagogastric cancers who are not able to undergo a traditional biopsy, multigene panel testing (MGPT) 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."⁴⁵

The guideline on non-small cell lung cancer (NSCLC) states the following in their section on molecular and biomarker analysis:⁴⁶

- "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. Molecular testing of tissue may have a higher yield in the setting of metastatic disease confined to the thorax.
- 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.
- At time of progression, consider ctDNA and tissue-based testing in parallel to identify resistance mechanisms.
- 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."

The guideline on Merkel cell carcinoma states: "The use of ctDNA in MCC has shown high disease sensitivity and prognostic accuracy for early detection of MCC recurrence. Based on NCCN panel expertise, it is recommended that additional tissue be harvested if a core biopsy is performed as there may be insufficient tissue remaining for a tissue-informed ctDNA test. This would negate the usefulness of ctDNA testing for continued surveillance of disease burden. Unlike traditional ctDNA testing, tissue-informed ctDNA has higher sensitivity as it utilizes a patients' tumor tissue as a baseline to indicate specific somatic mutations for assay

personalization. However, there are no current recommendations for treatment options following a positive ctDNA result; therefore, additional studies are required to utilize ctDNA surveillance effectively for patient care".⁴⁷

Note: Testing for other genes/variants may become accepted standard-of-care between policy updates. Blue Cross Blue Shield of Michigan and Blue Care Network follow National Comprehensive Cancer Network (NCCN) 2A or higher recommendations. Due to frequent NCCN updates, please refer to the appropriate guideline for the most recent standards and intended coverage. https://www.nccn.org/guidelines/category_1#site

National Institute for Health and Care Excellence

In 2022, the NICE issued the following guidance on the use of circulating tumor DNA (ctDNA), specifically the Signatera test, in the body to detect the presence of molecular residual disease (MRD) from solid tumors.⁴⁸ The experts concluded "that there is not enough evidence to support routine use of the technology in the NHS."

U.S. Preventive Services Task Force Recommendations

Not applicable.

Ongoing and Unpublished Clinical Trials

Some 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			
NCT06854939	A Prospective Study Investigating the Relationship Between Minimal Residual Disease Detection, Monitoring Frequency, and Prognosis in Non-small Cell Lung Cancer Patients Eligible for Curative Treatment. (MRDSEEKER)	350	Sept 2029
NCT06666439	Longitudinal Tumor Burden Quantification Using Circulating Tumor DNA in Metastatic Lobular Breast Cancer (LBC-Monitor)	20	Oct 2026
NCT06663787	Personalized Detection of CtDNA for Patients with HER2-Positive Metastatic Breast Cancer Who Achieved Durable Response by Anti-HER2 Treatment (HER2 CR) (HER2 CR)	50	Dec 2026 (active not recruiting)
NCT05987241	Testing the Role of DNA Released from Tumor Cells into the Blood in Guiding the Use of Immunotherapy After Surgical Removal of the Bladder, Kidney, Ureter, and Urethra for Urothelial Cancer Treatment, MODERN Study	992	Sept 2030
NCT06450314	Decreasing Treatment for Metastatic HER2-Positive Breast Cancer with Undetectable Cancer Levels in Blood Tests. (HEROES)	170	Dec 2029
NCT04920032 ^a	Proof of Concept Study of ctDNA Guided Change in Treatment for Refractory Minimal Residual Disease in Colon Adenocarcinomas	22	Dec 2026

NCT05081024 ^a	Establishing a ctDNA Biomarker to Improve Organ Preserving Strategies in individuals With Rectal Cancer	50	Sep 2026 (recruiting)
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
NCT05965479	Developing ctDNA Guided Adjuvant Therapy for Gastroesophageal Cancer (DECIPHER)	25	Apr 2028
NCT05914792	Longitudinal ctDNA Surveillance for Older Women With ER+ Breast Cancer Who Omit Surgery	40	July 2026
NCT07214532 ^a	Signatera-Guided Initiation of Adjuvant CDK4/6 Inhibitor in Intermediate Risk HR+ HER2- Breast Cancer	725	Dec 2037
NCT07125729	ctDNA Testing in Resectable Stage II-IV Colorectal Cancer Patients: A Head-to-Head Performance Comparison	150	Jan 2028
NCT07209215	ULtra sensiTive ctDNA-Informed Management eArly-stage recTal canCEr (ULTIMATE)	200	May 2028
NCT07084558	ABCSG 61 / TEODOR (Neoadjuvant Treatment Optimization Driven by ctDNA and endocrine Responsiveness): A Prospective, Randomized, Controlled, Open label Multicenter Phase II Study Investigating Neoadjuvant Endocrine Therapy Versus Chemotherapy in HR-positive, HER2negative, ctDNA-negative and Endocrine Responsive Early and Locally Advanced Breast Cancer	350	June 2033
NCT07058012	A Phase II Platform Study to Evaluate Treatment with Cemiplimab Monotherapy or Cemiplimab Plus Fianlimab and other Novel Combinations in Patients with Colorectal Cancer with Minimal Residual Disease Following Definitive Surgery and Chemotherapy	79	June 2028
NCT07282912	Phase II Prospective, Open Label Randomized Controlled Trial Comparing Standard of Care Therapy with and Without Sequential Cytoreductive Intervention for Patients with Metastatic Foregut Adenocarcinoma and Undetectable Circulating Tumor-Deoxyribose Nucleic Acid (ctDNA) Levels	54	June 2028
NCT07459673	HERizon-Breast: A ctDNA-Guided Adaptive Study of Sequential Anti-HER2 Therapies and CNS Prophylaxis to Induce Long-Term Remission	60	Mar 2030
Unpublished			
NCT05212779	Predicting the Risk of Ovarian Cancer Recurrence Using Circulating Tumor DNA to Assess Residual Disease	45	Dec 2024 (unknown status)
NCT04264702 ^a	BESPOKE Study of ctDNA Guided Therapy in Colorectal Cancer	2000	Sept 2025 (unknown status)

NCT: national clinical trial.

^a Denotes industry-sponsored or cosponsored trial.

Regulatory Status

Regulatory Status

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

Government Regulations

National:

There is no NCD for this testing.

Local:

Article A58468, effective 03/12/2026. Billing and Coding: MoIDX: Minimal Residual Disease Testing for Solid Tumor Cancers.

A Minimal Residual Disease (MRD) test, as defined in the related LCD, is a *service* that may include multiple components and/or multiple timepoints (i.e., serial assays). Testing schedules are set based on the validity established for the individual test comprising the service. Each component and timepoint of the service must be reasonable and necessary and adhere to the criteria set forth in the related LCD. The medical record must contain clear documentation of the medical necessity of this test, as per the conditions outlined in the LCD.

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

<u>Indicated Use</u>	<u>Test</u>
<u>PATIENTS WITH CANCER-</u> • <u>Initial Test</u>	
○ where tumor profiling is utilized from a prior analysis	SIGNATERA Bespoke Assay Design (by comprehensive genomic profile (CGP)) + Plasma Series Bundle for Molecular Residual Disease (Natera, Inc)
○ where tumor profiling is utilized	• SIGNATERA Bespoke Assay Design + Plasma Series Bundle for Molecular Residual Disease (Natera, Inc) • RaDaR Bespoke Assay Design + Plasma Series Bundle for Molecular Residual Disease (NeoGenomics Laboratories, Inc) • Oncodetect Bespoke Assay Design + Plasma Test Bundle (Exact Sciences Corp)
	• Guardant Reveal MRD Bundle (Guardant, Inc)

○ tumor naïve testing	• Guardant360 Response (Guardant, Inc)
• <u>Subsequent Test</u> ○ May only be performed if there is clinical evidence of a <i>priori</i> change in genetic content in accordance with NCD 90.2	
	• SIGNATERA <i>Recurrence Monitoring</i> Plasma Test Bundle (Natera, Inc) • Oncodetect Plasma Test Bundle (Exact Sciences Corp)
<u>PATIENTS WITHOUT CANCER (as defined in the policy) –</u>	
• <u>Initial Test</u>	
○ where tumor profiling is utilized - from a prior analysis	SIGNATERA <i>Recurrence Monitoring</i> Bespoke Assay Design (CGP) + single Plasma Test (Natera, Inc)
○ where tumor profiling is utilized	• SIGNATERA <i>Recurrence Monitoring</i> Bespoke Assay Design + single Plasma Test (Natera, Inc) • RaDaR <i>Recurrence Monitoring</i> Bespoke Assay Design + single Plasma Test (NeoGenomics Laboratories, Inc) • Oncodetect Bespoke Assay Design + single Plasma Test (Exact Sciences Corp) • Pathlight <i>Recurrence Monitoring</i> Bespoke Assay Design + single Plasma Test (SAGA Diagnostics) • NeXT Personal: WGS Assay Design + Plasma Initial Test (Personalis, Inc)
• <u>Subsequent Test</u>	• SIGNATERA <i>Recurrence Monitoring</i> single Plasma Test (Natera, Inc) • RaDaR <i>Recurrence Monitoring</i> single Plasma Test (NeoGenomics Laboratories, Inc) • Oncodetect single Plasma Test (Exact Sciences Corp) • NeXT Personal Single Plasma Test (Personalis, Inc) • Pathlight <i>Recurrence Monitoring</i> Bespoke Assay Design + single Plasma Test (SAGA Diagnostics)
• <u>Tumor Naïve Test</u> ○ Where baseline tumor profiling is not utilized	• NavDx single Plasma Test (Naveris, Inc) • Guardant Reveal single Plasma Test (Guardant, Inc)

Additional Test-specific Indications, Limitations and Instructions

- 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, NeXT Personal), advanced

breast cancer (NeXT Personal, Pathlight, RaDaR and Signatera) and HPV-driven oropharyngeal and anal squamous cell cancers (NavDx)

3. The monitoring of response to immune-checkpoint inhibitor therapy for advanced (Signatera) or inoperable/metastatic (Guardant Response) solid tumors.

However, the tests listed in the table may have only been approved for 1 or more (but not necessarily all) of these indications.

Note: 'Bundle' refers to multiple plasma assays.

(The above Medicare information is current as of the review date for this policy. However, the coverage issues and policies maintained by the Centers for Medicare & Medicare Services [CMS, formerly HCFA] are updated and/or revised periodically. Therefore, the most current CMS information may not be contained in this document. For the most current information, the reader should contact an official Medicare source.)

Related Policies

- Circulating Tumor DNA and Circulating Tumor Cells for Selecting Targeted Therapy for Advanced Solid Cancers (Liquid Biopsy)
- Somatic biomarker testing (including liquid biopsy) for targeted treatment in metastatic colorectal cancer (KRAS, NRAS, BRAF, NTRK, RET AND HER2)

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The articles reviewed in this research include those obtained in an Internet based literature search for relevant medical references through June 2026, the date the research was completed.

Joint BCBSM/BCN Medical Policy History

Policy Effective Date	BCBSM Signature Date	BCN Signature Date	Comments
11/1/26	8/18/26		Joint policy established

Next Review Date: 3rd Qtr., 2027

Pre-Consolidation Medical Policy History

Original Policy Date	Comments
BCN:	Revised:
BCBSM:	Revised:

BLUE CARE NETWORK BENEFIT COVERAGE
POLICY: GT-TUMOR-INFORMED CIRCULATING TUMOR DNA TESTING FOR CANCER
MANAGEMENT (E.G., SIGNATERA™)

I. Coverage Determination:

Commercial HMO (includes Self-Funded groups unless otherwise specified)	Not covered
BCNA (Medicare Advantage)	See government section
BCN65 (Medicare Complementary)	Coinsurance covered if primary Medicare covers the service.

II. Administrative Guidelines:

- The member's contract must be active at the time the service is rendered.
- Coverage is based on each member's certificate and is not guaranteed. Please consult the individual member's certificate for details. Additional information regarding coverage or benefits may also be obtained through customer or provider inquiry services at BCN.
- The service must be authorized by the member's PCP except for Self-Referral Option (SRO) members seeking Tier 2 coverage.
- Services must be performed by a BCN-contracted provider, if available, except for Self-Referral Option (SRO) members seeking Tier 2 coverage.
- Payment is based on BCN payment rules, individual certificate and certificate riders.
- Appropriate copayments will apply. Refer to certificate and applicable riders for detailed information.
- CPT - HCPCS codes are used for descriptive purposes only and are not a guarantee of coverage.