BSC2.18	Tumor-Informed Circulating Tumor DNA Testing for Cancer Management		
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Section:	2.0 Medicine	**Page:**	Page 1 of 50

Policy Statement

I. The use of a personalized, tumor-informed circulating tumor DNA (ctDNA) plasma-based test (e.g., Signatera by Natera or Personalized Cancer Monitoring—PCM by Invitae) for solid tumors is considered **medically necessary** when **BOTH** the following are met:
   A. Individual with stage I-IV cancer after surgical intervention with curative intent to provide information for **any** of the following:
      1. Adjuvant or targeted therapy
      2. Monitoring for relapse or progression (including but not limited to the use of immunotherapy immune checkpoint inhibitors {e.g., pembrolizumab [Keytruda], ipilimumab [Yervoy], nivilumab [Opdivo]})
   B. Frequency of testing does not exceed recommendations for monitoring noted in National Comprehensive Cancer Network (NCCN) guidelines for RECIST (Response Evaluation Criteria in Solid Tumors) for **any** of the following:
      1. Initial testing within 4-6 weeks after surgery as a baseline and for adjuvant therapy decisions
      2. Every 3-6 months for the first 2 years initially or with recurrence or progression (not to exceed 4 tests/year)
      3. Every 6-12 months for the following 3 years (not to exceed 2 tests/year) for colorectal cancer (CRC), NSCLC (Non-Small Cell Lung Cancer)
      4. Annually for the following 5 years (not to exceed 1 test/year)
      5. As indicated thereafter based on clinicopathologic features

II. The use of tumor-informed ctDNA is considered to be **investigational** for individuals with **any** of the following conditions:
   A. Pregnancy
   B. Active hematological malignancy
   C. History of allogeneic bone marrow/stem cell transplants
   D. Within 2 weeks after blood transfusion
   E. Other situations not meeting medically necessary criteria noted above

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

Policy Guidelines

This medical policy addresses the use of tumor-informed ctDNA MRD testing. The term MRD is variously used to describe Molecular, Minimal or Measurable Residual Disease. In this context, given the ctDNA testing used, MRD will be defined as Molecular Residual Disease.

Initial testing includes tumor testing (tumor block or FFPE slides from surgery or biopsy) and whole-blood testing (to allow matching of whole exome sequencing of tumor and blood DNA), and can take 35-42 days to complete. Subsequent monitoring testing is plasma only, based on initial testing results and is usually available within 7-14 days.

These tests are specifically designed for the detection of somatic mutations (in cancer) and are not appropriate for the identification of heritable germline mutations. This policy does not address the use of blood-based comprehensive genomic profile (CGP) or other testing to identify driver mutations to select targeted therapies or use of blood-based testing for gene expression profiling.

Refer to the following related Blue Shield of California Medical Policies for indications not covered in this policy:

- Circulating Tumor DNA and Circulating Tumor Cells for Cancer Management (Liquid Biopsy)
- Comprehensive Genomic Profiling for Selecting Targeted Cancer Therapies
- Gene Expression Profile Testing and Circulating Tumor DNA Testing for Predicting Recurrence in Colon Cancer
- Gene Expression Profiling and Protein Biomarkers for Prostate Cancer Management
- Genetic and Protein Biomarkers for the Diagnosis and Cancer Risk Assessment of Prostate Cancer
- Germline and Somatic Biomarker Testing (Including Liquid Biopsy) for Targeted Treatment and Immunotherapy in Breast Cancer
- Molecular Analysis (Including Liquid Biopsy) for Targeted Therapy or Immunotherapy of Non-Small-Cell Lung Cancer

This CPT code may be used for Tumor-informed circulating tumor DNA testing:

- **81479:** Unlisted molecular pathology procedure

Effective April 1, 2022 there are new CPT codes that represent PCM™ (Personalized Cancer Monitoring). Per the manufacturer, this test is patient- specific, tumor informed assay for the detection of circulating tumor DNA in the plasma of patients previously diagnosed with cancer.

- **0306U:** Oncology (minimal residual disease [MRD]), next-generation targeted sequencing analysis, cell-free DNA, initial (baseline) assessment to determine a patient-specific panel for future comparisons to evaluate for MRD
- **0307U:** Oncology (minimal residual disease [MRD]), next-generation targeted sequencing analysis of a patient-specific panel, cell-free DNA, subsequent assessment with comparison to previously analyzed patient specimens to evaluate for MRD

Effective October 1, 2022 there is a new CPT code that represents Signatera™. Per the manufacturer, this test is patient- specific, tumor informed assay for the detection of circulating tumor DNA in the plasma of patients previously diagnosed with cancer.

- **0340U:** Oncology (pan-cancer), analysis of minimal residual disease (MRD) from plasma, with assays personalized to each patient based on prior next-generation sequencing of the patient's tumor and germline DNA, reported as absence or presence of MRD, with disease-burden correlation, if appropriate

Description

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

NGS-based MRD assays can be either tumor-informed or tumor-agnostic

- Tumor-informed assays begin with a surgical specimen to determine which tumor-derived variants are present and are most amenable to tracking in cell-free DNA. That information can then be used to design a targeted NGS panel unique to the patient's tumor that can be used for subsequent monitoring.
- Tumor-agnostic assays include fixed panels designed to detect a specific number of genomic and/or epigenomic alterations commonly associated with a particular tumor type.

To detect MRD, tumor-informed and tumor-agnostic assays both rely on how well a tumor sheds DNA into the bloodstream, but tumor-informed approaches have the potential benefit of predicting which unique variants in a patient's tumor are the best alterations to detect and monitor. In contrast to the analysis of tumor biopsy samples, which are not only invasive to obtain but often also do not fully capture tumor heterogeneity and evolution, the analysis of ctDNA offers a non-invasive method of repeatedly evaluating the genomic profile of a patient's cancer to monitor for relapse, progression, treatment decisions, etc..

First used as a clinical metric in hematological cancers, MRD was historically measured with cell-based assays such as flow cytometry. Only recently, with the development of highly sensitive methods of DNA analysis, has ctDNA in plasma samples been shown to be a reliable biomarker for MRD for solid tumors as well.

Circulating tumor DNA (ctDNA) is a component of cell-free DNA that is shed by malignant tumors into the bloodstream and other bodily fluids[1]. Mechanisms of release into the bloodstream include cellular apoptosis, necrosis, phagocytosis, and active secretion[2-5]. ctDNA has clinically demonstrated the ability to function as a highly sensitive and specific cancer biomarker6-8. Quantitative characterization of ctDNA via liquid biopsy is associated with clinical and pathologic features of cancer, including stage, tumor burden, vascularization, and response to therapy[6-8.] The short half-life of ctDNA ensures that its detection captures tumor burden in real-time[9-10]. Crucial to its clinical role in guiding therapeutic decision-making, the presence of ctDNA is identified as molecular residual disease (MRD) which is clinically occult micrometastatic disease that remains in the patient during and after cancer treatment11-12. An overwhelming amount of literature suggests that MRD is subclinical disease responsible for cancer recurrence. Quantitative measures of ctDNA via a peripheral blood sample has been associated with a high-risk for recurrence but cannot be measured by standard clinical imaging techniques or other tumor biomarkers[13-15].

ctDNA serves as a biomarker for (1) postoperative and post-adjuvant chemotherapy treatment (ACT) risk stratification, (2) monitoring ACT effectiveness, (3) detection of clinical actionable mutations, and (4) early detection of recurrence. These observations have clinical implications and potential net health benefits for postoperative ctDNA-guided management of certain cancers.

Clinicopathologic risk factors have, and continue to dictate the patient selection strategy for adjuvant chemotherapy (ACT) in early-stage colorectal (CRC) patients. However, accumulating data indicate that the risk-stratification based on standard criteria is imprecise in identifying patients with MRD, resulting in overtreatment and undertreatment of a significant number of patients. In fact, approximately 50% of the stage III CRC patients and 74% of low-risk stage III CRC patients are cured by surgery alone. However, current guidelines recommend ACT for all such patients resulting in unnecessary chemotherapy administration in a large proportion of patients, causing a myriad of short- and long-term toxicities. Conversely, 5% of stage I CRC and 10-25% of Stage II patients recur; however, for Stage I and average-risk stage II patients, there is no available risk-stratification tool to identify patients who are likely to recur after curative surgery. Standard methods (e.g., RECIST or Response Evaluation Criteria in Solid Tumors) for monitoring disease after CRC surgery include radiological imaging and analysis of circulating tumor markers such as carcinoembryonic antigen (CEA) and serum cancer antigen 125 (CA-125). However, standard imaging detects only macroscopic disease, and the sensitivity and specificity of CEA and CA-125 are poor. Therefore, a biomarker that can identify earlier and more reliably patients with MRD is of critical importance in refining patient selection for adjuvant therapy.

Related Policies

- Circulating Tumor DNA and Circulating Tumor Cells for Cancer Management (Liquid Biopsy)
- Comprehensive Genomic Profiling for Selecting Targeted Cancer Therapies

- Gene Expression Profile Testing and Circulating Tumor DNA Testing for Predicting Recurrence in Colon Cancer
- Gene Expression Profiling and Protein Biomarkers for Prostate Cancer Management
- Genetic and Protein Biomarkers for the Diagnosis and Cancer Risk Assessment of Prostate Cancer
- Germline and Somatic Biomarker Testing (Including Liquid Biopsy) for Targeted Treatment and Immunotherapy in Breast Cancer
- Molecular Analysis (Including Liquid Biopsy) for Targeted Therapy or Immunotherapy of Non-Small-Cell Lung Cancer

Benefit Application

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

Some state or federal mandates (e.g., Federal Employee Program [FEP]) prohibits plans from denying Food and Drug Administration (FDA)-approved technologies as investigational. In these instances, plans may have to consider the coverage eligibility of FDA-approved technologies on the basis of medical necessity alone.

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 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 Breakthrough Device Designations covering new intended uses.[1,]

Rationale

Background

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

In addition to colorectal cancer (CRC), MRD testing has been performed in multiple cancer types. Validation studies for the use of MRD testing have been done for lung, breast, bladder, melanoma, cervical and esophageal cancers, among others. MRD testing across multiple cancer types demonstrates consistent sensitivity and specificity (ranging from 88-100% sensitivity and 98-100% specificity). Although the size, parameters, type of cancer and specific tests used for various studies varies, the results are consistent. Moreover, in these studies, ctDNA has shown significant lead time over radiographic imaging for the detection of relapse, and better performance than other standard indicators. In TRAcking non-small cell lung cancer (NSCLC) Evolution through therapy (TRACERx), a prospective study the median interval between ctDNA detection and detection of relapse by imaging was 70 days (range 10 to 346 days); in some of these cases, lead times of more than 6 months were noted.

Another longitudinal study in breast cancer patients found that plasma ctDNA was detected before clinical or radiologic relapse in 16 of 18 relapsed patients; in addition, ctDNA predicted metastatic relapse with a lead time of up to 2 years. A prospective study evaluating ctDNA before and after surgery and during chemotherapy in patients with locally advanced bladder cancer found that the measurement of ctDNA during treatment is a good predictor of outcome and a better predictor of treatment efficacy than pathologic downstaging (a commonly used surrogate marker). Moreover, in this study, patients without clearance of ctDNA had a response rate of 0%. ctDNA has also been shown to accurately monitor the activity and diagnose recurrence of endometrial cancer. Multiple studies have found it to be highly sensitive for monitoring and predicting disease progression and response to therapy in patients with metastatic melanoma.

Immune check point inhibitors (ICI) have emerged as an effective therapy and have been approved for various types of solid tumor malignancies. However, in most settings only a minority of patients respond to immunotherapy. FDA labels for ICI therapies call for treatment until disease progression or unacceptable toxicity. However, there is no definitive guidance on the method for evaluation of disease progression, which leaves this decision up to the judgement of clinicians prescribing these drugs, often without reliable objective measurements.

The determination as to whether a tumor is progressing is currently based largely on repeated radiographic evaluation of the tumor (sometimes combined with other biomarkers). While tumor growth is often associated with progression, this is complicated by pseudo-progression, where immune cell infiltration may cause the tumor to initially appear larger on a scan prior to shrinking, making it difficult to know in a timely fashion who is responding to treatment and who is not based on radiographic imaging and complicating patient management.

A recent (2022) study related to the use of ctDNA found that a ctDNA-guided approach compared to standard clinicopathological features for the treatment of stage II colon cancer reduced adjuvant chemotherapy use without compromising recurrence-free survival.

MRD testing using ctDNA is not without limitations and challenges in interpretation. There are limited studies reporting management changes made in response to ctDNA test results or net health outcomes based solely on ctDNA results, including long term outcomes. The sample sizes are small for some studies for certain cancers or for a particular test. And standard evaluations, management and treatments vary by type of cancer, meaning that test results need to be assessed with those differences in mind. Yet when considered in aggregate, the results are consistent and compelling. Because of the limitations noted, testing is currently most likely to be used in addition to standard approaches until further evidence emerges. However, given the significant limitations of current monitoring standards, there is still a place for ctDNA to add to the information available to clinicians for critical decision making.

Signatera (Natera) and Personalized Cancer Monitoring (PCM, Invitae)
Both tests are customized tumor-specific ctDNA test. Tumor tissue obtained from either a diagnostic biopsy or surgically resected tissue is used to identify 16 (for Signatera) or 18-50 (for PCM) 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 those 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 a few of these variants detected above a predefined confidence threshold are deemed to be 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 tests are currently intended to be used in conjunction with radiological assessment in most cases.

Literature Review

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

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

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

Indirect evidence on clinical utility rests on clinical validity. If the evidence is insufficient to demonstrate test performance, no inferences can be made about clinical utility.

Colorectal Cancer (CRC)

Clinical Context and Test Purpose

The purpose of tumor-informed ctDNA testing in individuals who have colorectal cancer is to inform treatment decisions and to monitor for recurrence following curative treatment.

The question addressed in this evidence review is: Does tumor-informed circulating tumor DNA (ctDNA) testing improve the net health outcome in individuals with colorectal cancer?

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

Populations

The relevant populations of interest are individuals:

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

Interventions

The test being considered is ctDNA testing with Signatera or PCM:

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

Comparators

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

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

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

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

Outcomes

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

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

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

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

Study Selection Criteria

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

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

Trials

Two randomized and nine nonrandomized studies examined the association of tumor-informed ctDNA testing to prognosis, DFS, recurrence, ctDNA dynamics and treatment response in individuals with colorectal cancer (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. Nine studies evaluated the association of ctDNA results (positive and negative) and risk for recurrence (disease free survival) in CRC (Table 2). Five studies reveal ctDNA results for early relapse detection as compared to CT Imaging (Table 3). Clinical findings from all 11 studies demonstrate clinical utility benefits for risk stratification for therapy selection, monitoring response to therapy, or early relapse detection in patients with resected CRC (table 4).

Randomized Trials

Tie, et al (2022) conducted an Australian trial to assess whether a ctDNA-guided approach could reduce the use of adjuvant chemotherapy without compromising recurrence risk. The study **enrolled 455 patients (322 for ctDNA and 153 standard) with medial followup of 37 months. About half of the ctDNA group got adjuvant therapy compared to standard (15 vs 28%). Yet ctDNA guided management was similar to standard for 2 year recurrence-free survival (93.5 vs. 92.4%).** Three-year recurrence-free survival was 86.4% among ctDNA-**positive** patients who received adjuvant

chemotherapy and 92.5% among ctDNA-**negative** patients who did not. The study authors concluded "A ctDNA-guided approach to the treatment of stage II colon cancer reduced adjuvant chemotherapy use without compromising recurrencefree survival."

Nonrandomized Trials

Four nonrandomized studies, 3 of which were noncomparative, examined the association of Signatera testing to prognosis in individuals with colorectal cancer (CRC) (Table 1). They differed in their study designs, populations (e.g., stage of disease), frequency and timing of standard care, outcome measures, and timing of follow up. Three studies evaluated the association between positive ctDNA results and prognosis in CRC (Table 2). These studies did not provide comparisons of ctDNA testing to standard methods of risk stratification for therapy selection, monitoring response to therapy, or early relapse detection. One retrospective study compared Signatera testing to other surveillance strategies in individuals with resected colorectal cancer.[2,] There are limited randomized controlled trials, and studies in which Signatera testing was used to guide treatment decisions.

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

Henriksen et al (2022) assessed the added benefit of serial ctDNA analysis; with samples taken at diagnosis, following surgery, during adjuvant therapy, and at follow up.[4,]

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.[5,] The study included an analysis of the sensitivity of Signatera testing to digital droplet 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).[2,] 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.

Tie et al (2016) investigated whether serial postsurgical and postchemotherapy ctDNA analysis could provide a real-time indication of adjuvant therapy efficacy in stage III colon cancer. This multicenter Australian trial recruited 100 consecutive patients (4 were excluded) with newly diagnosed stage III colon cancer. Median duration of follow-up was 28.9 months (range, 11.6-46.4 months). Main outcomes and measures included detection of ctDNA and recurrence-free interval (RFI). At least 1 somatic mutation was identified in the tumor tissue of all 96 evaluable patients. Circulating tumor DNA was detectable in 20 of 96 (21%) postsurgical samples and was associated with inferior recurrence-free survival (hazard ratio [HR], 3.8; 95% CI, 2.4-21.0; P < .001). Circulating tumor DNA was detectable in 15 of 88 (17%) postchemotherapy samples. The estimated 3-year RFI was 30% when ctDNA was detectable after chemotherapy and 77% when ctDNA was undetectable (HR, 6.8; 95% CI, 11.0-157.0; P < .001). Postsurgical ctDNA status remained independently associated with RFI after adjusting for known clinicopathologic risk factors (HR, 7.5; 95% CI, 3.5-16.1; P < .001). The authors concluded "Results suggest that ctDNA analysis after surgery is a promising prognostic marker in stage III colon cancer. Postchemotherapy ctDNA analysis may define a patient subset that remains at high risk of recurrence despite completing standard adjuvant treatment. This high-risk population presents a unique opportunity to explore additional therapeutic approaches."

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. Tumor-Informed ctDNA Testing Studies Supporting the Value of ctDNA for Molecular Residual Disease Assessment and Surveillance in Patients with Resected Colorectal Cancer.

Study	Major Findings	Conclusions & Relevance
Reinert et al[16]	At postoperative day 30, ctDNA-positive patients were 7 times more likely to relapse than ctDNA-negative patients (hazard ratio [HR], 7.2; 95% CI, 2.7-19.0; *P*< .001). After ACT ctDNA-positive patients were 17 times (HR, 17.5; 95% CI, 5.4-56.5; *P*< .001) more likely to relapse. During surveillance after definitive therapy, ctDNA-positive patients were more than 40 times more likely to experience disease recurrence than ctDNA-negative patients (HR, 43.5; 95% CI, 9.8-193.5 *P*< .001). In all multivariate analyses, ctDNA status was independently associated with relapse after adjusting for known clinicopathologic risk factors. Serial ctDNA analyses revealed disease recurrence up to 16.5 months ahead of standard-of-care radiologic imaging	The study reported that longitudinal ctDNA analysis in patients with stages I to III CRC can effectively detect and monitor changes in tumor burden throughout the clinical disease course Results show that ctDNA serves as a robust biomarker for (1) postoperative and post-ACT risk stratification, (2) monitoring ACT effectiveness, (3) detection of clinical actionable mutations, and (4) early detection of recurrence. The study found that in multivariate analysis ctDNA status (among stage, CEA, and other high-risk factors) was the only significant factor associated with recurrence. This suggests that ctDNA analysis may be a better tool for identifying high risk patients. The study provides first-line evidence that ACT can reduce the risk of recurrence in ctDNA-positive patients.
Henriksen et al[19]	Detection of ctDNA was a strong recurrence predictor postoperatively [HR = 7.0; 95% confidence interval (CI), 3.7–13.5; P < 0.001] and directly after ACT (HR = 50.76; 95% CI, 15.4–167; P < 0.001). Serial ctDNA assessment after the end of treatment was similarly predictive of recurrence (HR = 50.80; 95% CI, 14.9–172; P<0.001) The recurrence rate of postoperative ctDNA-positive patients treated with ACT was 80%. Only patients who cleared ctDNA permanently during ACT did not relapse. Serial ctDNA analysis every 3 months detected recurrence with a median lead-time of 9.8 months compared with standard-of-care computed tomography	The current study demonstrated ctDNA as a strong prognostic marker immediately post-ACT. The results suggest that radiological surveillance may be deescalated in low-risk (ctDNA-negative) patients with no/minimal effect on the outcome. For high-risk (ctDNA-positive) patients, there is an opportunity for intensifying imaging immediately upon ctDNA detection. The analysis comparing concurrent ctDNA and CT imaging assessments showed that in 33% of patients, who later recurred, ctDNA was detected at a time where no recurrence was visible by CT imaging. This indicates that ctDNA measurements in some cases may be more sensitive for recurrence detection than standard CT imaging ctDNA was the strongest prognostic marker in multivariable analysis with conventionally used risk markers. The findings are consistent with and extend on previous colorectal cancer studies
Tie et al[12]	Patients with ctDNA-positive status postoperatively had a markedly reduced	The study has demonstrated that stage II colon cancer patients who were ctDNA-

Study	Major Findings	Conclusions & Relevance
	recurrence-free survival (RFS) compared to those with a ctDNA-negative status [HR, 18; 95% confidence interval (CI), 7.9 to 40; P = 2.6 × 10−12]. Kaplan-Meier estimates of RFS at 3 years were 0% for the ctDNA-positive and 90% for the ctDNA-negative groups. Postoperative ctDNA status had a greater impact on RFS than any individual clinicopathological risk factor or any combination of clinicopathological factors. Postoperative ctDNA status added significant prognostic value to patients classified as either low-risk or high-risk on the basis of clinicopathological factors (low-risk: HR, 28; 95% CI, 8.1 to 93; P = 9.2 × 10−8; high-risk: HR, 7.5; 95% CI, 2.6 to 22; P = 0.0002). After multivariable adjustment, postoperative ctDNA status remained an independent predictor of RFS for patients not treated with chemotherapy (HR, 28; 95% CI, 11 to 68) and for all patients (HR, 14; 95% CI, 6.8 to 28) ctDNA positivity immediately after completion of chemotherapy was associated with poorer RFS (HR, 11; 95% CI, 1.8 to 68; P = 0.001) The median lead time from ctDNA detection to radiological recurrence was over 5 months, which might be sufficient to change patient management.	positive postoperatively are at extremely high risk of radiologic recurrence (HR, 18; 95% CI, 7.9 to 40; P = 2.6 × 10−12) when not treated with chemotherapy. Conversely, patients with negative ctDNA postoperatively were at a low risk of radiologic recurrence (3-year RFS of 90%), not dissimilar to patients with stage I colorectal cancer, defining a group where adjuvant therapy is less likely to be helpful. The prognostic impact of postoperative ctDNA status was independent of individual clinicopathological risk features and improved the RFS risk estimates for both patients with clinicopathologic low (HR, 28; 95% CI, 8.1 to 93) and high-risk features (HR, 7.5; 95% CI, 2.6 to 22). The study also demonstrated that being ctDNA-positive at the completion of adjuvant chemotherapy treatment predicted a very high risk of radiologic recurrence. The findings that postoperative ctDNA is a robust predictor of disease recurrence is consistent with recent reports in other tumor types. This ctDNA measurement is superior to clinicopathological measures currently used to guide adjuvant chemotherapy decisions. ctDNA detection after stage II colon cancer resection provides direct evidence of residual disease and identifies patients at very high risk of recurrence.
Tie et al[11]	Significantly worse recurrence-free survival was seen if ctDNA was detectable after chemoradiotherapy (HR 6.6; P<0.001) or after surgery (HR 13.0; P<0.001). The estimated 3-year recurrence-free survival was 33% for the postoperative ctDNA -positive patients and 87% for the postoperative ctDNA -negative patients. Postoperative ctDNA detection was predictive of recurrence irrespective of adjuvant chemotherapy use (chemotherapy: HR 10.0; P<0.001; without chemotherapy: HR 22.0; P<0.001) Postoperative ctDNA status remained an independent predictor of recurrence-free survival after adjusting for known clinicopathological risk factors (HR 6.0; P<0.001).	This study provides the first evidence that circulating tumor DNA analysis after curative intent surgery for locally advanced rectal cancer could stratify patients into subsets at very high risk or low risk of recurrence. The strong prognostic impact of postoperative circulating tumor DNA status appears to be independent of other known pathological risk factors.

Study	Major Findings	Conclusions & Relevance
Tarazona et al[20]	Detection of ctDNA after surgery and in serial plasma samples during follow-up were associated with poorer disease-free survival (DFS) [hazard ratio (HR), 17.56; log-rank P = 0.0014 and HR, 11.33; log-rank P = 0.0001, respectively]. In patients treated with adjuvant chemotherapy, presence of ctDNA after therapy was associated with early relapse (HR 10.02; log-rank P < 0.0001). Detection of ctDNA at follow-up preceded radiological recurrence with a median lead time of 11.5 months. ctDNA was the only significantly independent predictor of DFS in multivariable analysis.	Plasma postoperative ctDNA detected MRD and identified patients at high risk of relapse in localized CC. The data showed the presence of postoperative ctDNA establishes a stronger prognostic marker than conventional pathological factors such as stage. The findings are consistent with previous studies, which have already shown that ctDNA after surgery can be a biomarker of MRD and therefore a prognostic factor for recurrence in patients with colorectal cancer.
Tie et al[21]	Patients with detectable postoperative ctDNA experienced a significantly lower RFS (HR 6.3; 95% CI 2.58 to 15.2; P < 0.001) and overall survival (HR 4.2; 95% CI 1.5 to 11.8; P < 0.001) compared to patients with undetectable ctDNA All patients with detectable postoperative ctDNA who failed to clear their ctDNA following adjuvant chemotherapy experienced recurrence, while 67% of patients whose ctDNA became undetectable after chemotherapy remained disease-free End-of-treatment (surgery +/− adjuvant chemotherapy) ctDNA detection was associated with a 5-year RFS of 0% compared to 75.6% for patients with an undetectable end-of-treatment ctDNA (HR 14.9; 95% CI 4.94 to 44.7; P < 0.001).	We confirmed the prognostic impact of post-surgery and post-treatment ctDNA in patients with resected CRLM. The potential utility of serial ctDNA analysis during adjuvant chemotherapy as an early marker of treatment efficacy was also demonstrated ctDNA detection after surgery or after completion of adjuvant chemotherapy is associated with a very high risk of recurrence and death in patients with resectable CRLM ctDNA dynamics before and after adjuvant chemotherapy reflected adjuvant treatment efficacy.
Loupakis et al[22]	Postsurgical, MRD positivity was observed in 54.4% (61 of 112) of patients, of which 96.7% (59 of 61) progressed at the time of data cutoff (hazard ratio [HR]: 5.8; 95% CI, 3.5 to 9.7; P < .001). MRD-positive status was also associated with an inferior overall survival: HR: 16.0; 95% CI, 3.9 to 68.0; P < .001. At the time of analyses, 96% (49 of 51) of patients were alive in the MRD-negative arm compared with 52.4% (32 of 61) in the MRD-positive arm. Patients who did not receive systemic therapy and were MRD-negative in the combined ctDNA analysis at two time points had an overall survival of 100%.	This study confirms that in mCRC undergoing resection of metastases, postoperative MRD analysis is a strong prognostic biomarker The present work supports the continuous expansion of the number of clinical studies in patients with mCRC using personalized ctDNA-based MRD analysis and provides direct evidence of the predictive and prognostic value of ctDNA, which could help clinicians and researchers with real numbers to design their clinical studies and support therapeutic decisions in the adjuvant setting

Study	Major Findings	Conclusions & Relevance
	In the multivariate analysis, ctDNA-based MRD status was the most significant prognostic factor associated with disease-free survival (HR: 5.78; 95% CI, 3.34 to 10.0; P < .001).	
Fakih et al[23]	Surveillance with CEA measurement appeared to perform poorly in detecting a first recurrence, with a sensitivity of only 20.0% (95% CI, 5.3%-48.6%). Circulating tumor DNA did not appear to perform numerically better than imaging, with sensitivities of 53.3% (95% CI, 27.4%-77.7%) and 60.0% (95% CI, 32.9%-82.5%), (P > .99), respectively. The specificity was the highest for ctDNA at 100% (95% CI, 87.0%-100%) When combining imaging and measurement of CEA levels, as recommended by NCCN guidelines, the combination modality had a numerical advantage compared with ctDNA in identifying a recurrence (sensitivity, 73.3% [95% CI, 44.8%-91.1%]; P = .55) and performed well on both the PPV (73.3% [95% CI, 44.8%-91.1%] vs 100% [95% CI, 59.8%-100%]) and NPV (87.9% [95% CI, 70.9%- 96.0%] vs 82.5% [95% CI, 66.6%-92.1%]). Statistical analysis showed that the sensitivity of CEA surveillance was significantly worse than that of combined imaging and measurement of CEA levels (20.0% [95% CI, 5.3%-48.6%]; P = .01). No significant difference was noted among ctDNA (median, 14.3 months), imaging (median, 15.0 months), or imaging plus measurement of CEA levels (median, 15.0 months) in the time to identify disease recurrence.	The findings of this prospective cohort study suggest that ctDNA assay may not provide definitive advantages as a surveillance strategy compared with standard imaging combined with measurement of CEA levels when performed per NCCN guidelines. However, a positive ctDNA finding without doubt indicates an almost definitive risk of relapse.
Tie et al.[26]	Circulating tumor DNA was detectable in 20 of 96 (21%) postsurgical samples and was associated with inferior recurrence-free survival (hazard ratio [HR], 3.8; 95% CI, 2.4-21.0; P < .001) In this multicenter cohort study of 96 patients with stage III colon cancer, a significant difference in 3-year recurrence-free interval was observed in patients with detectable vs undetectable levels of circulating tumor DNA after surgery (47% vs 76%) The estimated 3-year RFI was 30% when ctDNA was detectable after chemotherapy and 77% when ctDNA was undetectable (HR, 6.8; 95% CI, 11.0-157.0; P < .001)	Results suggest that ctDNA analysis after surgery is a promising prognostic marker in stage III colon cancer. Post chemotherapy ctDNA analysis may define a patient subset that remains at high risk of recurrence despite completing standard adjuvant treatment.

Study	Major Findings	Conclusions & Relevance
	Postsurgical ctDNA status remained independently associated with RFI after adjusting for known clinicopathologic risk factors (HR, 7.5; 95% CI, 3.5-16.1; P < .001).	
Tie et al.[24]	455 patients underwent randomization, 302 were assigned to ctDNA-guided management and 153 to standard of care management A lower percentage of patients in the ctDNA-guided group than in the standard-management group received adjuvant chemotherapy (15% vs. 28%; relative risk, 1.82; 95% confidence interval [CI], 1.25 to 2.65). In the evaluation of 2-year recurrence-free survival, ctDNA-guided management was noninferior to standard management (93.5% and 92.4%, respectively; absolute difference, 1.1 percentage points; 95% CI, −4.1 to 6.2 [noninferiority margin, −8.5 percentage points]). Three-year recurrence-free survival was 86.4% among ctDNA-positive patients who received adjuvant chemotherapy and 92.5% among ctDNA-negative patients who did not. Among ctDNA-negative patients, 3-year recurrence-free survival was higher among patients with clinical low-risk cancers than among those with high-risk cancers (96.7% vs. 85.1%; hazard ratio, 3.04; 95% CI, 1.26 to 7.34) 3-year recurrence-free survival was higher among patients with T3 tumors than among those with T4 tumors (94.2% vs. 81.3%; hazard ratio, 2.60; 95% CI, 1.01 to 6.71)	In this trial, we found that a ctDNA-guided approach reduced the number of patients who received adjuvant therapy and did not alter the risk of recurrence. ctDNA positive patients appeared to derive considerable benefit from adjuvant treatment, given the low percentage of patients with recurrence in this trial as compared with previously reported high recurrence rates in this subgroup of patients when no adjuvant chemotherapy was administered The study confirmed the very low risk of recurrence in untreated ctDNA-negative patient Most notable was the 3-year recurrence-free survival of 96.7% among patients with low-risk disease, indicating that adjuvant therapy should not be considered for ctDNA negative patients who are at clinicopathological low risk. This is an important observation, because in routine clinical practice adjuvant chemotherapy is still administered to some patients at low risk (11% in our standard-management group), particularly younger patients The results of this trial suggest that a survival benefit from adjuvant chemotherapy may be obtained in a well-defined subgroup of patients with stage II colon cancer — namely, those with detectable ctDNA after surgery A ctDNA-guided approach to the treatment of stage II colon cancer reduced adjuvant chemotherapy use without compromising recurrence-free survival.
Kotaka et al[25]	GALAXY enrolled 1,564 patients, of whom 1,040 (the "outcome cohort") were included in the current analysis. Patients who tested positive for ctDNA 4 weeks after surgery had an 11- to 13-times increased risk for recurrence. The assay's sensitivity for disease recurrence was calculated at 63.6% for the analysis of patients with stage I to IV disease and 67.6% for those with stage II to III disease.	The study shows that stratifying post-surgical treatment decisions using the ctDNA assay can identify patients likely to benefit from ACT across all stages of disease, including pStage II.

Study	Major Findings	Conclusions & Relevance
	The multivariate analysis showed the risk of recurrence for patients with stage II to III tumors was highly correlated with ctDNA positivity at 4 weeks after surgery (hazard ratio [HR] = 15.3; *P* < .001). Patients with baseline ctDNA positivity who remained positive over the course of treatment had an almost 16-fold increased risk of disease recurrence Patients who did not clear their ctDNA between 4- and 12-weeks during adjuvant chemotherapy had significantly worse outcomes relative to those who cleared their ctDNA—i.e., "positive to positive" (58.3%) vs "positive to negative" (100%)—exhibiting a 15.8-fold increased risk. The disease-free survival rate for ctDNA-negative patients that remained negative was 98% and for those who turned positive 62.5%. For patients with high-risk stage II, stage III, and stage IV disease, adjuvant chemotherapy yielded a benefit among patients who tested positive for ctDNA 4 weeks after surgery, with hazard ratios of 9.4 (*P* = .04), 8.8 (*P* < .001), and 2.4 (*P* = .02), respectively. Patients with high-risk stage II to III disease who tested negative for ctDNA at 4 weeks, on the other hand, had excellent outcomes, whether or not they received chemotherapy, with a disease-free survival of approximately 95% at 12 months.	

HR: hazard ratio, ACT: adjuvant chemotherapy, DSF: disease free survival, RFS: recurrence free survival, CRLM: colorectal cancer liver metastases, mCRC: metastatic colorectal cancer

Table 2. Recurrence Rates by Risk Category (ctDNA Positive/Negative) in Tumor-Informed ctDNA Testing Studies in Colorectal Cancer

Study	Recurrence Risk After Surgery Study Population	Recurrence Risk After ACT	Longitudinal Recurrence Risk
Reinert et al[3]	At postoperative day 30, ctDNA-positive patients were 7 times more likely to relapse than ctDNA-negative patients (hazard ratio [HR], 7.2; 95% CI, 2.7-19.0; *P* < .001).	After ACT ctDNA-positive patients were 17 times (HR, 17.5; 95% CI, 5.4-56.5; *P* < .001) more likely to relapse.	During surveillance after definitive therapy, ctDNA-positive patients were more than 40 times more likely to experience disease recurrence than ctDNA-negative patients (HR, 43.5; 95% CI, 9.8-193.5 *P* < .001).
Henriksen et al[4]	Detection of ctDNA was a strong recurrence predictor postoperatively [HR = 7.0; 95% confidence interval (CI), 3.7–13.5; P < 0.001]	Detection of ctDNA was a strong predictor of recurrence after ACT (HR = 50.76; 95% CI, 15.4–167; P < 0.001).	Serial ctDNA assessment after the end of treatment was similarly predictive of recurrence (HR = 50.80; 95% CI, 14.9–172; P < 0.001)

Study	Recurrence Risk After Surgery Study Population	Recurrence Risk After ACT	Longitudinal Recurrence Risk
Tie et al[29]	Patients with ctDNA-positive status postoperatively had a markedly reduced recurrence-free survival (RFS) compared to those with a ctDNA-negative status [HR = 18; 95% CI, 7.9 to 40; P = 2.6×10^{-12}]	ctDNA positivity immediately after completion of chemotherapy was associated with poorer RFS (HR, 11; 95% CI, 1.8 to 68; P = 0.001)	The prognostic impact of postoperative ctDNA status was independent of individual clinicopathological risk features and improved the RFS risk estimates for both patients with clinicopathologic low (HR = 28; 95% CI, 8.1 to 93) and high-risk features (HR = 7.5; 95% CI, 2.6 to 22).
Tie et al[27]	Significantly worse recurrence-free survival was seen if ctDNA was detectable after surgery (HR = 13.0; P<0.001)	Significantly worse recurrence-free survival was seen if ctDNA was detectable after chemoradiotherapy (HR = 6.6; P<0.001)	Postoperative ctDNA detection was predictive of recurrence irrespective of adjuvant chemotherapy use (chemotherapy: HR = 10.0; P<0.001; without chemotherapy: HR = 22; P<0.001). Postoperative ctDNA status remained an independent predictor of recurrence-free survival after adjusting for known clinicopathological risk factors (HR = 6.0; P<0.001).
Tarazona et al[24]	The presence of ctDNA immediately after surgery was associated with poorer DFS (HR 6.96; P = 0.0001). After multivariable adjustment, postoperative ctDNA status remained the only significant predictor of DFS (HR 11.64; 95% CI 3.67-36.88; P < 0.001)	ctDNA positivity after completion of chemotherapy was associated with poorer DFS (HR 10.02; 95% CI 9.202 - 307.3; P < 0.0001)	Detection of ctDNA after surgery and in serial plasma samples during follow-up were associated with poorer DFS [HR 17.56; log-rank P = 0.0014 and HR 11.33; log-rank P = 0.0001, respectively].
Tie et al[30]	Patients with detectable postoperative ctDNA experienced a significantly lower RFS (HR 6.3; 95% CI 2.58 to 15.2; P < 0.001) and overall survival (HR 4.2; 95% CI 1.5 to 11.8; P < 0.001) compared to patients with undetectable ctDNA	The estimated 5-year RFS was 66.7% for patients who cleared their ctDNA after adjuvant chemotherapy compared to 0% in patients with persistently positive ctDNA after adjuvant chemotherapy (HR, 7.87; 95% CI 0.95 to 63.7; P = 0.056)	End-of-treatment (surgery +/− adjuvant chemotherapy) ctDNA detection was associated with a 5-year RFS of 0% compared to 75.6% for patients with an undetectable end-of-treatment ctDNA (HR 14.9; 95% CI 4.94 to 44.7; P < 0.001).
Loupakis et al[5]	Postsurgical, MRD positivity was observed in 54.4% (61 of 112) of patients, of which 96.7% (59 of 61) progressed at the time of data cutoff (HR: 5.8; 95% CI, 3.5 to 9.7; P < .001).	At the time of analyses, 96% (49 of 51) of patients were alive in the MRD-negative arm compared with 52.4% (32 of 61) in the MRD-positive arm. Patients who did not receive systemic therapy and were MRD-negative in the combined ctDNA analysis at two time points had an overall survival of 100%.	ctDNA-based MRD status was the most significant prognostic factor associated with disease-free survival (HR: 5.78; 95% CI, 3.34 to 10.0; P < .001).
Tie et al[26]	Circulating tumor DNA was detectable in 20 of 96 (21%)	The estimated 3-year RFI was 30% when ctDNA was	Postsurgical longitudinal ctDNA status remained

Study	Recurrence Risk After Surgery Study Population	Recurrence Risk After ACT	Longitudinal Recurrence Risk
	postsurgical samples and was associated with inferior recurrence-free survival (hazard ratio [HR], 3.8; 95% CI, 2.4-21.0; P < .001).	detectable after chemotherapy and 77% when ctDNA was undetectable (HR, 6.8; 95% CI, 11.0-157.0; P < .001).	independently associated with RFI after adjusting for known clinicopathologic risk factors (HR, 7.5; 95% CI, 3.5-16.1; P < .001).
Tie et al[31]	N/A	A lower percentage of patients in the ctDNA-guided group than in the standard-management group received adjuvant chemotherapy (15% vs. 28%; relative risk, 1.82; 95% confidence interval [CI], 1.25 to 2.65). In the evaluation of 2-year recurrence-free survival, ctDNA-guided management was noninferior to standard management (93.5% and 92.4%, respectively; absolute difference, 1.1 percentage points; 95% CI, −4.1 to 6.2 [noninferiority margin, −8.5 percentage points]).	The estimated 3-year recurrence-free survival was 92.5% among ctDNA-negative patients and 86.4% among ctDNA-positive patients (HR 1.83; 95% CI, 0.79 to 4.27)
Kotaka et al[32]	N/A	6-month post operative DFS in overall population (pStage I-IV) for ctDNA negative patients was 96.5% (95.0-97.5; 95% CI), while 6-month DFS in ctDNA positive patients was 62.8% (55.4-69.2; 95%CI).	12-month post operative DFS in overall population (pStage I-IV) for ctDNA negative patients was 92.7% (90.4-94.5; 95% CI), while 12-month DFS in ctDNA positive patients was 47.5% (39.3-55.2; 95%CI; HR 10.9; P<0.001).

ACT: adjuvant chemotherapy; HR: hazard ratio; CI: confidence interval; RFS: recurrence free survival; RFI: recurrence free interval

Table 3. Tumor-Informed ctDNA Testing Compared to Other Surveillance Strategies in Resected Colorectal Cancer

Study	Sensitivity	Specificity	PPV	NPV	Median Time to Recurrence, Months
Reinert et al[3]	ctDNA 88% CEA 69%	ctDNA 98% CEA 64%	N/A	N/A	The mean lead time from ctDNA detection in plasma to relapse detection by standard-of-care computed tomography was 8.7 months (range, 0.8-16.5 months; Wilcoxon signed rank test; P<.001); by contrast CEA revealed no lead time.
Henriksen et al[4]	N/A	N/A	N/A	N/A	Serial ctDNA analysis every 3 months detected recurrence with a median lead-time of 9.8 months compared with standard-of-care computed tomography. (IQR: 2–9; P < 0.001, Wilcoxon signed rank test)
Tie et al[29]	ctDNA 48%	ctDNA 100%	N/A	N/A	85% of patients were ctDNA-positive up to or at the time of radiologic recurrence, whereas

Study	Sensitivity	Specificity	PPV	NPV	Median Time to Recurrence, Months
					CEA was only elevated in 41% of patients. The median lead time from ctDNA detection to radiological recurrence was over 5 months
Tarazona et al[24]	N/A	N/A	N/A	N/A	Detection of ctDNA had a median of 11.5 months (range 3-18 months) lead time over radiological relapse
Loupakis et al[5]	ctDNA 91.4% CEA 46%	ctDNA 93.3%	ctDNA 96.7%	N/A	N/A
Fakih et al[2]	ctDNA 53.3% Imaging 60% CEA 20% CEA + Imaging 73.3%	ctDNA 100% Imaging 96.9% CEA 90.9% CEA + Imaging 87.9%	ctDNA 100% Imaging 90% CEA 50% CEA + Imaging 73.3%	ctDNA 82.5% Imaging 84.2% CEA 71.4% CEA + Imaging 87.9%	Median time to recurrence (months) ctDNA 14.3 Imaging 15.0 CEA N/A CEA + Imaging 15.0

CEA: carcinoembryonic antigen; CRC: colorectal cancer; CT: computerized tomography; IQR: interquartile range; NCCN: National Comprehensive Cancer Network.

Table 4. Tumor-Informed ctDNA Testing Studies Supporting the Value of ctDNA for Molecular Residual Disease Assessment and Surveillance in Patients with Resected Colorectal Cancer.

Study	Major Findings	Conclusions & Relevance
Reinert et al[3]	At postoperative day 30, ctDNA-positive patients were 7 times more likely to relapse than ctDNA-negative patients (hazard ratio [HR], 7.2; 95% CI, 2.7-19.0; *P*< .001). After ACT ctDNA-positive patients were 17 times (HR, 17.5; 95% CI, 5.4-56.5; *P*< .001) more likely to relapse. During surveillance after definitive therapy, ctDNA-positive patients were more than 40 times more likely to experience disease recurrence than ctDNA-negative patients (HR, 43.5; 95% CI, 9.8-193.5 *P*< .001). In all multivariate analyses, ctDNA status was independently associated with relapse after adjusting for known clinicopathologic risk factors. Serial ctDNA analyses revealed disease recurrence up to 16.5 months ahead of standard-of-care radiologic imaging	The study reported that longitudinal ctDNA analysis in patients with stages I to III CRC can effectively detect and monitor changes in tumor burden throughout the clinical disease course Results show that ctDNA serves as a robust biomarker for (1) postoperative and post-ACT risk stratification, (2) monitoring ACT effectiveness, (3) detection of clinical actionable mutations, and (4) early detection of recurrence. The study found that in multivariate analysis ctDNA status (among stage, CEA, and other high-risk factors) was the only significant factor associated with recurrence. This suggests that ctDNA analysis may be a better tool for identifying high risk patients. The study provides first-line evidence that ACT can reduce the risk of recurrence in ctDNA-positive patients.
Henriksen et al[4]	Detection of ctDNA was a strong recurrence predictor postoperatively [HR = 7.0; 95% confidence interval (CI), 3.7–13.5; P < 0.001] and directly after ACT (HR = 50.76; 95% CI, 15.4–167; P < 0.001). Serial ctDNA assessment after the end of treatment was similarly predictive of	The current study demonstrated ctDNA as a strong prognostic marker immediately post-ACT. The results suggest that radiological surveillance may be deescalated in low-risk (ctDNA-negative) patients with no/minimal effect on the outcome. For high-risk (ctDNA-positive) patients, there is an opportunity for

Study	Major Findings	Conclusions & Relevance
	recurrence (HR = 50.80; 95% CI, 14.9–172; P<0.001) The recurrence rate of postoperative ctDNA-positive patients treated with ACT was 80%. Only patients who cleared ctDNA permanently during ACT did not relapse. Serial ctDNA analysis every 3 months detected recurrence with a median lead-time of 9.8 months compared with standard-of-care computed tomography	intensifying imaging immediately upon ctDNA detection. The analysis comparing concurrent ctDNA and CT imaging assessments showed that in 33% of patients, who later recurred, ctDNA was detected at a time where no recurrence was visible by CT imaging. This indicates that ctDNA measurements in some cases may be more sensitive for recurrence detection than standard CT imaging ctDNA was the strongest prognostic marker in multivariable analysis with conventionally used risk markers. The findings are consistent with and extend on previous colorectal cancer studies
Tie et al[29]	Patients with ctDNA-positive status postoperatively had a markedly reduced recurrence-free survival (RFS) compared to those with a ctDNA-negative status [HR, 18; 95% confidence interval (CI), 7.9 to 40; P = 2.6 × 10−12]. Kaplan-Meier estimates of RFS at 3 years were 0% for the ctDNA-positive and 90% for the ctDNA-negative groups. Postoperative ctDNA status had a greater impact on RFS than any individual clinicopathological risk factor or any combination of clinicopathological factors. Postoperative ctDNA status added significant prognostic value to patients classified as either low-risk or high-risk on the basis of clinicopathological factors (low-risk: HR, 28; 95% CI, 8.1 to 93; P = 9.2 × 10−8; high-risk: HR, 7.5; 95% CI, 2.6 to 22; P = 0.0002). After multivariable adjustment, postoperative ctDNA status remained an independent predictor of RFS for patients not treated with chemotherapy (HR, 28; 95% CI, 11 to 68) and for all patients (HR, 14; 95% CI, 6.8 to 28) ctDNA positivity immediately after completion of chemotherapy was associated with poorer RFS (HR, 11; 95% CI, 1.8 to 68; P = 0.001) The median lead time from ctDNA detection to radiological recurrence was over 5 months, which might be sufficient to change patient management.	The study has demonstrated that stage II colon cancer patients who were ctDNA-positive postoperatively are at extremely high risk of radiologic recurrence (HR, 18; 95% CI, 7.9 to 40; P = 2.6 × 10−12) when not treated with chemotherapy. Conversely, patients with negative ctDNA postoperatively were at a low risk of radiologic recurrence (3-year RFS of 90%), not dissimilar to patients with stage I colorectal cancer, defining a group where adjuvant therapy is less likely to be helpful. The prognostic impact of postoperative ctDNA status was independent of individual clinicopathological risk features and improved the RFS risk estimates for both patients with clinicopathologic low (HR, 28; 95% CI, 8.1 to 93) and high-risk features (HR, 7.5; 95% CI, 2.6 to 22). The study also demonstrated that being ctDNA-positive at the completion of adjuvant chemotherapy treatment predicted a very high risk of radiologic recurrence. The findings that postoperative ctDNA is a robust predictor of disease recurrence is consistent with recent reports in other tumor types. This ctDNA measurement is superior to clinicopathological measures currently used to guide adjuvant chemotherapy decisions. ctDNA detection after stage II colon cancer resection provides direct evidence of residual disease and identifies patients at very high risk of recurrence.
Tie et al[27]	Significantly worse recurrence-free survival was seen if ctDNA was detectable after	This study provides the first evidence that circulating tumor DNA analysis after curative

Study	Major Findings	Conclusions & Relevance
	chemoradiotherapy (HR 6.6; P<0.001) or after surgery (HR 13.0; P<0.001). The estimated 3-year recurrence-free survival was 33% for the postoperative ctDNA -positive patients and 87% for the postoperative ctDNA -negative patients. Postoperative ctDNA detection was predictive of recurrence irrespective of adjuvant chemotherapy use (chemotherapy: HR 10.0; P<0.001; without chemotherapy: HR 22.0; P<0.001) Postoperative ctDNA status remained an independent predictor of recurrence-free survival after adjusting for known clinicopathological risk factors (HR 6.0; P<0.001).	intent surgery for locally advanced rectal cancer could stratify patients into subsets at very high risk or low risk of recurrence. The strong prognostic impact of postoperative circulating tumor DNA status appears to be independent of other known pathological risk factors.
Tarazona et al[24]	Detection of ctDNA after surgery and in serial plasma samples during follow-up were associated with poorer disease-free survival (DFS) [hazard ratio (HR), 17.56; log-rank P = 0.0014 and HR, 11.33; log-rank P = 0.0001, respectively]. In patients treated with adjuvant chemotherapy, presence of ctDNA after therapy was associated with early relapse (HR 10.02; log-rank P < 0.0001). Detection of ctDNA at follow-up preceded radiological recurrence with a median lead time of 11.5 months. ctDNA was the only significantly independent predictor of DFS in multivariable analysis.	Plasma postoperative ctDNA detected MRD and identified patients at high risk of relapse in localized CC. The data showed the presence of postoperative ctDNA establishes a stronger prognostic marker than conventional pathological factors such as stage. The findings are consistent with previous studies, which have already shown that ctDNA after surgery can be a biomarker of MRD and therefore a prognostic factor for recurrence in patients with colorectal cancer.
Tie et al[30]	Patients with detectable postoperative ctDNA experienced a significantly lower RFS (HR 6.3; 95% CI 2.58 to 15.2; P < 0.001) and overall survival (HR 4.2; 95% CI 1.5 to 11.8; P < 0.001) compared to patients with undetectable ctDNA All patients with detectable postoperative ctDNA who failed to clear their ctDNA following adjuvant chemotherapy experienced recurrence, while 67% of patients whose ctDNA became undetectable after chemotherapy remained disease-free End-of-treatment (surgery +/− adjuvant chemotherapy) ctDNA detection was associated with a 5-year RFS of 0% compared to 75.6% for patients with an undetectable end-of-treatment ctDNA (HR 14.9; 95% CI 4.94 to 44.7; P < 0.001).	We confirmed the prognostic impact of post-surgery and post-treatment ctDNA in patients with resected CRLM. The potential utility of serial ctDNA analysis during adjuvant chemotherapy as an early marker of treatment efficacy was also demonstrated ctDNA detection after surgery or after completion of adjuvant chemotherapy is associated with a very high risk of recurrence and death in patients with resectable CRLM ctDNA dynamics before and after adjuvant chemotherapy reflected adjuvant treatment efficacy.

Study	Major Findings	Conclusions & Relevance
Loupakis et al[5]	Postsurgical, MRD positivity was observed in 54.4% (61 of 112) of patients, of which 96.7% (59 of 61) progressed at the time of data cutoff (hazard ratio [HR]: 5.8; 95% CI, 3.5 to 9.7; P < .001). MRD-positive status was also associated with an inferior overall survival: HR: 16.0; 95% CI, 3.9 to 68.0; P < .001. At the time of analyses, 96% (49 of 51) of patients were alive in the MRD-negative arm compared with 52.4% (32 of 61) in the MRD-positive arm. Patients who did not receive systemic therapy and were MRD-negative in the combined ctDNA analysis at two time points had an overall survival of 100%. In the multivariate analysis, ctDNA-based MRD status was the most significant prognostic factor associated with disease-free survival (HR: 5.78; 95% CI, 3.34 to 10.0; P < .001).	This study confirms that in mCRC undergoing resection of metastases, postoperative MRD analysis is a strong prognostic biomarker The present work supports the continuous expansion of the number of clinical studies in patients with mCRC using personalized ctDNA-based MRD analysis and provides direct evidence of the predictive and prognostic value of ctDNA, which could help clinicians and researchers with real numbers to design their clinical studies and support therapeutic decisions in the adjuvant setting
Fakih et al[2]	Surveillance with CEA measurement appeared to perform poorly in detecting a first recurrence, with a sensitivity of only 20.0% (95% CI, 5.3%-48.6%). Circulating tumor DNA did not appear to perform numerically better than imaging, with sensitivities of 53.3% (95% CI, 27.4%-77.7%) and 60.0% (95% CI, 32.9%-82.5%), (P > .99), respectively. The specificity was the highest for ctDNA at 100% (95% CI, 87.0%-100%) When combining imaging and measurement of CEA levels, as recommended by NCCN guidelines, the combination modality had a numerical advantage compared with ctDNA in identifying a recurrence (sensitivity, 73.3% [95% CI, 44.8%-91.1%]; P = .55) and performed well on both the PPV (73.3% [95% CI, 44.8%-91.1%] vs 100% [95% CI, 59.8%-100%]) and NPV (87.9% [95% CI, 70.9%- 96.0%] vs 82.5% [95% CI, 66.6%-92.1%]). Statistical analysis showed that the sensitivity of CEA surveillance was significantly worse than that of combined imaging and measurement of CEA levels (20.0% [95% CI, 5.3%-48.6%]; P = .01). No significant difference was noted among ctDNA (median, 14.3 months), imaging (median, 15.0 months), or imaging plus measurement of CEA levels (median, 15.0	The findings of this prospective cohort study suggest that ctDNA assay may not provide definitive advantages as a surveillance strategy compared with standard imaging combined with measurement of CEA levels when performed per NCCN guidelines. However, a positive ctDNA finding without doubt indicates an almost definitive risk of relapse.

Study	Major Findings	Conclusions & Relevance
	months) in the time to identify disease recurrence.	
Tie et al.[26]	Circulating tumor DNA was detectable in 20 of 96 (21%) postsurgical samples and was associated with inferior recurrence-free survival (hazard ratio [HR], 3.8; 95% CI, 2.4-21.0; P < .001) In this multicenter cohort study of 96 patients with stage III colon cancer, a significant difference in 3-year recurrence-free interval was observed in patients with detectable vs undetectable levels of circulating tumor DNA after surgery (47% vs 76%) The estimated 3-year RFI was 30% when ctDNA was detectable after chemotherapy and 77% when ctDNA was undetectable (HR, 6.8; 95% CI, 11.0-157.0; P < .001) Postsurgical ctDNA status remained independently associated with RFI after adjusting for known clinicopathologic risk factors (HR, 7.5; 95% CI, 3.5-16.1; P < .001).	Results suggest that ctDNA analysis after surgery is a promising prognostic marker in stage III colon cancer. Post chemotherapy ctDNA analysis may define a patient subset that remains at high risk of recurrence despite completing standard adjuvant treatment.
Tie et al.[31]	455 patients underwent randomization, 302 were assigned to ctDNA-guided management and 153 to standard of care management A lower percentage of patients in the ctDNA-guided group than in the standard-management group received adjuvant chemotherapy (15% vs. 28%; relative risk, 1.82; 95% confidence interval [CI], 1.25 to 2.65). In the evaluation of 2-year recurrence-free survival, ctDNA-guided management was noninferior to standard management (93.5% and 92.4%, respectively; absolute difference, 1.1 percentage points; 95% CI, −4.1 to 6.2 [noninferiority margin, −8.5 percentage points]). Three-year recurrence-free survival was 86.4% among ctDNA-positive patients who received adjuvant chemotherapy and 92.5% among ctDNA-negative patients who did not. Among ctDNA-negative patients, 3-year recurrence-free survival was higher among patients with clinical low-risk cancers than among those with high-risk cancers (96.7% vs. 85.1%; hazard ratio, 3.04; 95% CI, 1.26 to 7.34) 3-year recurrence-free survival was higher among patients with T3 tumors than among those with T4 tumors (94.2% vs. 81.3%; hazard ratio, 2.60; 95% CI, 1.01 to 6.71)	In this trial, we found that a ctDNA-guided approach reduced the number of patients who received adjuvant therapy and did not alter the risk of recurrence. ctDNA positive patients appeared to derive considerable benefit from adjuvant treatment, given the low percentage of patients with recurrence in this trial as compared with previously reported high recurrence rates in this subgroup of patients when no adjuvant chemotherapy was administered The study confirmed the very low risk of recurrence in untreated ctDNA-negative patient Most notable was the 3-year recurrence-free survival of 96.7% among patients with low-risk disease, indicating that adjuvant therapy should not be considered for ctDNA negative patients who are at clinicopathological low risk. This is an important observation, because in routine clinical practice adjuvant chemotherapy is still administered to some patients at low risk (11% in our standard-management group), particularly younger patients The results of this trial suggest that a survival benefit from adjuvant chemotherapy may be obtained in a well-defined subgroup of patients with stage II colon cancer —

Study	Major Findings	Conclusions & Relevance
		namely, those with detectable ctDNA after surgery A ctDNA-guided approach to the treatment of stage II colon cancer reduced adjuvant chemotherapy use without compromising recurrence-free survival.
Kotaka et al[32]	GALAXY enrolled 1,564 patients, of whom 1,040 (the "outcome cohort") were included in the current analysis. Patients who tested positive for ctDNA 4 weeks after surgery had an 11- to 13-times increased risk for recurrence. The assay's sensitivity for disease recurrence was calculated at 63.6% for the analysis of patients with stage I to IV disease and 67.6% for those with stage II to III disease. The multivariate analysis showed the risk of recurrence for patients with stage II to III tumors was highly correlated with ctDNA positivity at 4 weeks after surgery (hazard ratio [HR] = 15.3; $P < .001$). Patients with baseline ctDNA positivity who remained positive over the course of treatment had an almost 16-fold increased risk of disease recurrence Patients who did not clear their ctDNA between 4- and 12-weeks during adjuvant chemotherapy had significantly worse outcomes relative to those who cleared their ctDNA—i.e., "positive to positive" (58.3%) vs "positive to negative" (100%)—exhibiting a 15.8-fold increased risk. The disease-free survival rate for ctDNA-negative patients that remained negative was 98% and for those who turned positive 62.5%. For patients with high-risk stage II, stage III, and stage IV disease, adjuvant chemotherapy yielded a benefit among patients who tested positive for ctDNA 4 weeks after surgery, with hazard ratios of 9.4 ($P = .04$), 8.8 ($P < .001$), and 2.4 ($P = .02$), respectively. Patients with high-risk stage II to III disease who tested negative for ctDNA at 4 weeks, on the other hand, had excellent outcomes, whether or not they received chemotherapy, with a disease-free survival of approximately 95% at 12 months.	The study shows that stratifying post-surgical treatment decisions using the ctDNA assay can identify patients likely to benefit from ACT across all stages of disease, including pStage II.

HR: hazard ratio, ACT: adjuvant chemotherapy, DSF: disease free survival, RFS: recurrence free survival, CRLM: colorectal cancer liver metastases, mCRC: metastatic colorectal cancer

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)[3,]	1. individual selection not described					Multiple subgroup analyses, small numbers of individuals with positive ctDNA tests.
Henriksen et al (2022)[4,]			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)[5,]						Small numbers of individuals with positive ctDNA tests.
Fakih et al (2022)[2,]						

The study limitations stated in this table are those notable in the current review; this is not a comprehensive gaps assessment.
ctDNA: circulating tumor DNA.
[a] Selection key: 1. Selection not described; 2. Selection not random or consecutive (ie, 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 colorectal cancer (CRC) who receive tumor-informed ctDNA testing with Signatera to guide treatment decisions and monitor for recurrence, the evidence includes 3 noncomparative studies (N = 410) and 1 retrospective comparative study (N = 48).

Nonrandomized studies have reported an association between ctDNA results measured at diagnosis, following surgery, during adjuvant therapy, and during surveillance after curative treatment and prognosis, but these studies are limited by a lack of comparison to tests used for the same purpose, imprecise estimates due to small sample sizes, and clinical heterogeneity of study populations. No study reported management changes made in response to ctDNA test results. A retrospective observational study found no advantage to surveillance with Signatera compared to standard surveillance conducted according to NCCN guidelines (p>.99 for sensitivity and specificity compared

to imaging). There is no direct evidence that the use of the test improves health outcomes, and indirect evidence is not sufficient to draw conclusions about clinical validity.

Breast Cancer

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

The question addressed in this evidence review is: Does tumor-informed circulating tumor DNA testing improve the net health outcome in individuals with breast cancer?

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

Populations

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

Interventions

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

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

Comparators

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

Outcomes

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

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

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

If used for disease surveillance following primary treatment, beneficial outcomes of a true positive test would be earlier detection of metastasis and initiation of treatment. Harmful outcomes of a false positive test would be undergoing unnecessary or incorrect treatment, and experiencing adverse effects of such treatment. See also Evidence review 2.04.36 for additional discussion of outcomes in breast cancer risk assessment studies.

Study Selection Criteria

For the evaluation of clinical validity of tumor informed ctDNA testing, studies that meet the following eligibility criteria were considered:

- Reported on the accuracy of the marketed version of the technology

- Included a suitable reference standard
- Individual/sample clinical characteristics were described
- Individual/sample selection criteria were described.

Nonrandomized Trials

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.[6,] 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.[7,] 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.

Coombes et al (2019), in a longitudinal study6 in breast cancer patients found that plasma ctDNA was detected before clinical or radiologic relapse in 16 of 18 relapsed patients; moreover, ctDNA predicted metastatic relapse with a lead time of up to 2 years.

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)[6,]	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)[7,]	Response to neoadjuvant chemotherapy	84 individuals with $\geq$ 2.5 cm nonmetastatic stage II/III breast cancer	Retrospective analysis of samples prospectively collected as part of the I-SPY2 TRIAL	Radiological imaging	2 or more variants detected out of 16	Plasma samples collected before, during, and after neoadjuvant chemotherapy	Yes

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

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

Study	Initial N	Final N	Excluded Samples	Recurrence Rate	Median Time to Recurrence, months (range)	Clinical Validity			
						Sensitivity	*Specificity*	*PPV*	*NPV*
Coombes et al (2019)[6,]	197	49	148	18/49 (36.7%)	8.9 (0.5 to 24.0)	16/18 (89%)	31/31 (100%)	NR	NR
Hazard ratio (95% CI) for RFS (first postsurgical sample)	11.8 (4.3 to 32.5), p<.001								
Hazard ratio (95% CI) for RFS (any follow up sample)	35.8 (7.9 to 161.3), p<.001								
Magbanua et al (2021)[7,]	84	75	9	NA	NA	NR	NR	4/6 (67%)	50/54 (93%)
Hazard ratio (95% CI) for recurrence (T0, baseline)	4.11 (0.52 to 32.4)								
Hazard ratio (95% CI) for RFS (T1, 3 weeks after therapy initiation)	4.5 (1.2 to 17.4)								
Hazard ratio (95% CI) for RFS (T2, between regimens)	5.4 (1.3 to 22.5)								
Hazard ratio (95% CI) for RFS (T3, after neoadjuvant chemotherapy)	11.5 (2.9 to 46.1)								

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

Table 8. Study Relevance Limitations

Study	Population[a]	Intervention[b]	Comparator[c]	Outcomes[d]	Duration of Follow-Up[e]
Coombes et al (2019)[6,]	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)[7,]			3. Not compared to tests used for		

Study	Population[a]	Intervention[b]	Comparator[c]	Outcomes[d]	Duration of Follow-Up[e]
			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)[6,]						1. Confidence intervals for test characteristics not reported; small number of positive ctDNA tests
Magbanua et al (2021)[7,]	2. Retrospective analysis					1. Confidence intervals for test characteristics not reported; small number of positive ctDNA tests

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 (ie, 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 in Individuals with Breast Cancer

For individuals with breast cancer who receive tumor-informed ctDNA testing to guide treatment decisions and monitor for recurrence, the evidence includes 2 noncomparative studies (N = 133). One study evaluated Signatera testing for disease surveillance following primary treatment, and 1 reported the association of test results at different timepoints with response to neoadjuvant chemotherapy. Although the studies found an association of test results with prognosis, the studies are limited by a lack of comparison to tests used for the same purpose, imprecise estimates due to small sample sizes, and clinical heterogeneity of study populations. No study reported management

changes made in response to ctDNA test results. There is no direct evidence that the use of the test improves health outcomes, and indirect evidence is not sufficient to draw conclusions about clinical validity.

Bladder Cancer

Clinical Context and Test Purpose

The purpose of testing in individuals with bladder cancer is to predict disease course to inform treatment decisions and to monitor for recurrence following curative treatment.

The question addressed in this evidence review is: Does tumor-informed circulating tumor DNA testing improve the net health outcome in individuals with bladder cancer?
The following PICO was used to select literature to inform this review.

Populations

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

Interventions

The test being considered is circulating tumor DNA testing:

- At diagnosis, to identify individuals at low risk of recurrence after cystectomy who may be eligible for cystectomy without neoadjuvant chemotherapy.
- After chemotherapy before cystectomy, to determine treatment response and inform treatment decisions (e.g., additional cycles of chemotherapy or other therapeutic strategies).
- During disease surveillance after cystectomy, to identify metastatic relapse after cystectomy at an early time point, and aid in the selection of individuals who may benefit from early/adjuvant treatment. For individuals with bladder cancer who are being evaluated for adjuvant chemotherapy, given that the test will be used to *rule-in* individuals for adjuvant chemotherapy, the performance characteristics of most interest are positive predictive value and specificity.

Comparators

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

Outcomes

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

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

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

Study Selection Criteria

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

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

Nonrandomized Trials

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

Christensen et al (2019) assessed the association of ctDNA with prognosis in 68 individuals with localized advanced bladder cancer.[8,]

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.[9,] Study participants who were positive for ctDNA had improved disease-free survival and overall survival in the atezolizumab arm versus the observation arm (disease-free survival hazard ratio = 0.58 [95% CI, 0.43–0.79]; p=.0024 and overall survival hazard ratio = 0.59 [95% CI, 0.41–0.86]). No difference in disease-free survival or overall survival between treatment arms was noted for patients who were negative for ctDNA.

Christensen et al (2019) A prospective study evaluating ctDNA before and after surgery and during chemotherapy in patients with locally advanced bladder cancer found that the dynamics of ctDNA during treatment is a good predictor of outcome and a better predictor of treatment efficacy than pathologic downstaging. Moreover, in this study, patients without clearance of ctDNA had a response rate of 0%8.

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
Christensen et al (2019) [8,]	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.	Yes
Powles et al (2021)[9,]	581 individuals with urothelial cancer from a randomized	Retrospective	Radiological imaging	greater or equal to 2 variants	Post-surgical plasma samples were collected and	No

Study	Study Population	Study Design and Setting	Reference Standard	Threshold for Positive Index Test	Timing of Reference and Index Tests	Blinding of Assessors
	Phase III trial of adjuvant atezolizumab vs. observation who had undergone surgery and were evaluable for ctDNA			detected out of 16	tested at baseline and 6 weeks after randomization and individuals were followed up for a median of 23 months	

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
Christensen et al (2019) [8,]		
At diagnosis before chemotherapy	11/24 (46%)	1/35 (3%)
Adjusted hazard ratio (95% CI) for recurrence	29.1; p=.001	
After chemotherapy before cystectomy	6/8 (75%)	6/55 (11%)
Adjusted hazard ratio (95% CI) for recurrence	12.0; p<.001	
During disease surveillance after cystectomy	13/17 (76%)	0/47 (0%)
Adjusted hazard ratio for recurrence	129.6; p<.001	
Powles et al (2021)[9,]		
Following surgery (cycle 1 day 1)		
Hazard ratio (95% CI) for DFS	6.3 (4.45 to 8.92); p<.0001	
6 weeks after randomization (cycle 3 day 1)		
Hazard ratio (95% CI) for DFS	8.65 (5.67 to 13.18); p<.0001	

CI: confidence interval; ctDNA: circulating tumor DNA; DFS: disease-free survival.

Table 12. Study Relevance Limitations

Study	Population[a]	Intervention[b]	Comparator[c]	Outcomes[d]	Duration of Follow-Up[e]
Christensen et al (2019) [8,]			3. Not compared to tests used for the same purpose		
Powles et al (2021)[9,]	.		3. Not compared to tests used for the same purpose		

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

Table 13. Study Design and Conduct Limitations

Study	Selection[a]	Blinding[b]	Delivery of Test[c]	Selective Reporting[d]	Data Completeness[e]	Statistical[f]
Christensen et al (2019) [8,]						1. Confidence intervals for hazard ratios not reported.
Powles et al (2021)[9,]						

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 (ie, 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 in Individuals with Bladder Cancer

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

Non-Small Cell Lung Cancer (NSCLC)

Clinical Context and Test Purpose

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

The question addressed in this evidence review is: Does tumor-informed circulating tumor DNA testing improve the net health outcome in individuals with NSCLC?

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

Populations

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

Interventions

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

Adjuvant platinum-based chemotherapy is not the standard of care following surgery for NSCLC; treatment improves cure rates after surgery in only 5% of patients, and 20% of patients receiving

chemotherapy experience acute toxicities. Testing is proposed to select patients who are very likely to relapse post-operatively and who might benefit from adjuvant treatment.

Comparators

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

Outcomes

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

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

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

Nonrandomized Trial

The evidence for the use of ctDNA testing 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.[10,] 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%).

Also, the TRAcking non-small cell lung cancer (NSCLC) Evolution through therapy (TRACERx), is a prospective study phylogenetically profiling and monitoring (from diagnosis to death) the clonal evolution of tumors in 100 NSCLC patients. The median interval between ctDNA detection and detection of relapse by imaging was 70 days (range 10 to 346 days); in some of these cases, lead times of more than 6 months were observed.[10] In some cases, further subclonal analysis revealed targetable mutations and amplification events implicated in driving the relapse, thereby also impacting the therapeutic options available to a given patient.[10]

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

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

Study	Study Population	Study Design and Setting	Reference Standard	Threshold for Positive Index Test	Timing of Reference and Index Tests	Blinding of Assessors	Main Results
Abbosh et al (2017)[10,]	24 individuals with early-stage NSCLC	Prospective, subgroup of patients enrolled in the	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	Yes	Of 14 individuals with confirmed relapse, 13

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
		TRACERx Study			thereafter; individuals were followed up for a median of 775 days		(93%) had a positive ctDNA test Of 10 individuals with no relapse after a median followup 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).

Table 15. Study Relevance Limitations

Study	Population[a]	Intervention[b]	Comparator[c]	Outcomes[d]	Duration of Follow-Up[e]
Abbosh et al (2017)[10,]			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)[10,]	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

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 in Individuals with Non-Small Cell Lung Cancer

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

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

Clinical Context and Test Purpose

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

The question addressed in this evidence review is: Does tumor-informed circulating tumor DNA testing improve the net health outcome in individuals with esophageal cancer?

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

Populations

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

Interventions

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

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

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

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

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

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

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

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

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

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

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

Study	Study Population	Study Design and Setting	Reference Standard	Threshold for Positive Index Test	Timing of Reference and Index Tests	Blinding of Assessors
Ococks et al (2021)[11],	17 individuals with esophageal adenocarcinoma who had undergone surgery	Retrospective	Radiological imaging	2 or more variants detected out of 16	Blood samples were collected before and after surgical treatment and patients were followed up for a median of 43.4 months.	Yes

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

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

ctDNA: circulating tumor DNA; NR: not reported; RFS: recurrence-free survival.

Table 19. Study Relevance Limitations

Study	Population[a]	Intervention[b]	Comparator[c]	Outcomes[d]	Duration of Follow-Up[e]
Ococks et al (2021)[11],		2. Unclear if the test used was the commercially available version	3. No comparison to tests used for the same purpose		

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

Table 20. Study Design and Conduct Limitations

Study	Selection[a]	Blinding[b]	Delivery of Test[c]	Selective Reporting[d]	Data Completeness[e]	Statistical[f]
Ococks et al (2021)[11],					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 in Individuals with Esophageal Cancer

For individuals with esophageal cancer who receive tumor-informed ctDNA testing to guide treatment decisions and monitor for recurrence, the evidence includes 1 noncomparative, retrospective study (N = 17). Patients who were ctDNA-positive before surgery had significantly poorer disease-free survival (DFS) (p<.042), with a median DFS of 32.0 months versus 63.0 months in ctDNA-negative preoperative patients. This study was limited by its small number sample size and retrospective design. There is no direct evidence that the use of the test improves health outcomes. Due to the study's limitations and lack of additional supporting studies, the evidence is not sufficient to draw conclusions on clinical validity. Additionally, the management pathway for Signatera testing in esophageal cancer has not been clearly defined.

Immunotherapy for Solid Tumors

Clinical Context and Test Purpose

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

The question addressed in this evidence review is: Does tumor-informed circulating tumor DNA testing improve the net health outcome in individuals with solid tumors who have received immunotherapy?

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

Populations

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

Interventions

The test being considered is circulating tumor DNA testing with Signatera.

Comparators

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

Outcomes

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

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

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

Study Selection Criteria

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

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

- Individual/sample selection criteria were described.

Nonrandomized Trial

Bratman et al (2020) evaluated Signatera to predict treatment response in 106 individuals receiving pembrolizumab for solid tumors, including squamous cell cancer of head and neck, triple negative breast cancer, high-grade serous ovarian cancer, malignant melanoma, and mixed solid tumors (Tables 21 and 22).[12,]

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 progression free survival (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)[12,]	106 individuals with advanced solid tumors who were enrolled in a Phase II clinical trial of pembrolizumab (NCT02644369)	Prospective, single center	TMB, PD-L1 testing, radiological imaging	Greater or equal to 2 variants detected out of 16	Baseline sample obtained and after every 3 cycles; individuals were followed up for a median of 25 months	Yes

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

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

	Overall Survival
Bratman et al (2020)[12,]	
Lower than median ctDNA at baseline	adjusted HR 0.49 (95% CI 0.29–0.83)
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; RFS: 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)[12,]	1, 2. Unclear what management		No comparison to standard	3. Clinical validity	

	changes would be implemented based on test results.	surveillance methods	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)[12,]						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 (ie, 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 in Individuals with Solid Tumors Receiving Immunotherapy

For individuals with solid tumors who receive tumor-informed ctDNA testing to monitor response to immunotherapy, the evidence includes a subgroup analysis of individuals enrolled in a nonrandomized trial of pembrolizumab (N = 106). The subgroup analysis evaluated 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 progression free survival (adjusted HR 0.54, 95% CI 0.34 to 0.85). The study was limited by a small sample size, variability in results across different tumor types, and lack of a comparison to standard methods of monitoring response to treatment. There is limited direct evidence that the use of the test improves health outcomes. The management pathway for tumor-informed ctDNA testing for monitoring response to immunotherapy needs further definition.

Other cancers

ctDNA has also been shown to accurately monitor the activity and diagnose recurrence of endometrial cancer[59], and multiple studies have found it to be highly sensitive for monitoring and predicting disease progression and response to therapy in patients with metastatic melanoma[60, 61].

Overall Summary of Evidence

For individuals with **CRC** who receive tumor-informed ctDNA testing to guide treatment decisions and monitor for recurrence, the evidence includes 3 noncomparative studies (N = 410) 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. Nonrandomized studies have reported an association between ctDNA results measured at diagnosis, following surgery, during adjuvant therapy, and during surveillance after curative treatment and prognosis, but these studies are limited by a lack of comparison to tests used for the same purpose, imprecise estimates due to small sample sizes, and clinical heterogeneity of study populations. No study reported management changes made in response to ctDNA test results. A retrospective observational study found no clear advantage to surveillance compared to standard surveillance conducted according to NCCN guidelines (p>.99 for sensitivity and specificity compared to imaging), however, direct use comparison was not done in the study. There is limited direct evidence that the use of the test improves health outcomes. However, indirect evidence supports the use of this technology.

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

For individuals with **bladder** cancer who receive tumor-informed ctDNA testing with Signatera to guide treatment decisions and monitor for recurrence, the evidence includes 1 uncontrolled prospective cohort study (N = 68) and 1 retrospective subgroup analysis from a RCT (N = 581). Relevant outcomes are overall survival, disease-specific survival, test validity, other test performance measure, change in disease status, morbid events, functional outcomes, health status measures, quality of life, and treatment-related mortality. The prospective study reported an association between test results at diagnosis, during chemotherapy treatment, and during surveillance following cystectomy to prognosis. The retrospective analysis reported an association between test results and response to atezolizumab treatment. Study limitations, including a lack of comparison to tests used for the same purpose limit drawing definitive conclusions about clinical validity and usefulness. No study reported management changes made in response to ctDNA test results. There is limited direct evidence that the use of the test improves health outcomes, however, indirect evidence supports the use of this technology.

For individuals with **NSCLC** who receive tumor-informed ctDNA testing to guide treatment decisions and monitor for recurrence, the evidence includes 1 subgroup analysis of participants enrolled in a prospective observational study (N = 24). Relevant outcomes are overall survival, disease-specific survival, test validity, other test performance measures, change in disease status, morbid events, functional outcomes, health status measures, quality of life, and treatment-related mortality. Of 14 individuals with confirmed relapse, 13 (93%) had a positive ctDNA test (defined as at least 2 single-nucleotide variants detected). Of 10 individuals with no relapse after a median follow up of 775 days, (range 688 to 945 days), 1 had a positive ctDNA test (10%). This study's small sample size and lack of a comparator limit drawing definitive conclusions about clinical validity. There is limited direct evidence

that the use of the test improves health outcomes, however, indirect evidence supports the use of this technology.

For individuals with **esophageal** cancer who receive tumor-informed ctDNA testing to guide treatment decisions and monitor for recurrence, the evidence includes 1 noncomparative, retrospective study (N = 17). Relevant outcomes are overall survival, disease-specific survival, test validity, other test performance measure, change in disease status, morbid events, functional outcomes, health status measures, quality of life, and treatment-related mortality. Patients who were ctDNA-positive before surgery had significantly poorer disease-free survival (DFS) (p<.042), with a median DFS of 32.0 months versus 63.0 months in ctDNA-negative preoperative patients. This study was limited by its small number sample size and retrospective design. There is limited direct evidence that the use of the test improves health outcomes. However, indirect evidence supports the use of this technology.

For individuals with solid tumors who receive tumor-informed ctDNA testing to monitor response to **immunotherapy**, the evidence includes a subgroup analysis of individuals enrolled in a nonrandomized trial of pembrolizumab (N = 106). Relevant outcomes are overall survival, disease-specific survival, test validity, other test performance measures, change in disease status, morbid events, functional outcomes, health status measures, quality of life, and treatment-related mortality. The subgroup analysis evaluated 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 progression free survival (adjusted HR 0.54, 95% CI 0.34 to 0.85). The study was limited by a small sample size, variability in results across different tumor types, and lack of a comparison to standard methods of monitoring response to treatment. There is limited direct evidence that the use of the test improves health outcomes. However, indirect evidence supports the use of this technology.

Supplemental Information

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

Practice Guidelines and Position Statements

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

National Comprehensive Cancer Network

National Comprehensive Cancer Network guidelines do not yet specifically address tumor-informed ctDNA testing for any of the cancer types included in this review. The guidelines on colon cancer state: "The panel believes that there are insufficient data to recommend the use of multigene assays, Immunoscore or post-surgical ctDNA to estimate risk of recurrence or determine adjuvant therapy."[13,

U.S. Preventive Services Task Force Recommendations

Not applicable.

Medicare National Coverage

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

Medicare Local Coverage Determination by MolDx allows for coverage, stating:

LCD - MolDX: Minimal Residual Disease Testing for Cancer (L38779) (cms.gov)

"MRD testing for cancer is rapidly becoming a sensitive and specific method for monitoring the relative amounts of tumor-derived genetic material circulating in the blood of cancer patients. These tests leverage new genomic technologies that allow detection of extremely dilute tumor material, yielding an extremely sensitive method for determining the continued presence of tumor material or, by serially testing the same individual, tracking the relative increase or decrease of tumor material being deposited in the blood. Although it is a relatively new application of novel genomic technologies, it has rapidly demonstrated its ability to impact patient care in several ways in cancer diagnosis and treatment. MRD testing can be used to:

- diagnose cancer progression, recurrence, or relapse before there is clinical, biological, or radiographical evidence of progression, recurrence or relapse
- detect tumor response to therapy by measuring the proportional changes in the amount of available tumor DNA

Both above uses may enable physicians to better assign risk stratification, deploy alternate treatment strategies, or preclude the use of unnecessary adjuvant therapies."

Ongoing and Unpublished Clinical Trials

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

Table 25. Summary of Key Trials

NCT No.	Trial Name	Planned Enrollment	Completion Date
Ongoing			
NCT05212779	Predicting the Risk of Ovarian Cancer Recurrence Using Circulating Tumor DNA to Assess Residual Disease	45	Dec 2024
NCT04761783[a]	BESPOKE Study of ctDNA Guided Immunotherapy	1539	May 2025
NCT04264702[a]	BESPOKE Study of ctDNA Guided Therapy in Colorectal Cancer	2000	Jan 2025
NCT04786600[a]	A Phase II Randomized Therapeutic Optimization Trial for Subjects With Refractory Metastatic Colorectal Cancer Using ctDNA: Rapid 1 Trial	78	May 2025
NCT05178576[a]	A Single Arm Phase II Study to Evaluate Treatment With Gevokizumab in individuals With Stage II/III Colon Cancer Who Are ctDNA-positive After Curative Surgery and Adjuvant Chemotherapy	31	Nov 2025
NCT04920032[a]	Proof of Concept Study of ctDNA Guided Change in Treatment for Refractory Minimal Residual Disease in Colon Adenocarcinomas	22	Jun 2024
NCT05060003[a]	A Phase II Randomized Study of Tiragolumab Plus Atezolizumab Versus Atezolizumab in the Treatment of Stage II Melanoma individuals Who Are ctDNA-positive Following Resection	244	Feb 2028
NCT05081024[a]	Establishing a ctDNA Biomarker to Improve Organ Preserving Strategies in individuals With Rectal Cancer	50	Sep 2024
NCT05067842	A Pilot Observational Study to Assess Feasibility of Tumor Response Assessment by Circulating Tumor DNA (ctDNA) in individuals With Locally Advanced Esophageal and GE Junction Adenocarcinoma Undergoing Treatment With Total Upfront Chemotherapy and Chemoradiation	30	Jan 2028
NCT04670588	A Prospective Observational Study to Determine the Feasibility of Tumor Response Assessment by Circulating Tumor DNA in individuals With Locally Advanced Rectal Cancer Undergoing Total Neoadjuvant Therapy	30	Dec 2025
NCT04929015	Peritoneal Carcinomatosis Leveraging ctDNA Guided Treatment in GI Cancer Study (PERICLES Study)	30	Nov 2024

NCT No.	Trial Name	Planned Enrollment	Completion Date
NCT05058183[a]	Safe De-escalation of Chemotherapy for Stage 1 Breast Cancer	400	Nov 2027
NCT05174169[a]	Colon Adjuvant Chemotherapy Based on Evaluation of Residual Disease	1912	Jan 2030

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

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Documentation for Clinical Review

Please provide the following documentation:

- History and physical and/or consultation notes including:

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

- Radiology report(s) and interpretation (i.e., MRI, CT, PET)

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

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

Coding

This Policy relates only to the services or supplies described herein. Benefits may vary according to product design; therefore, contract language should be reviewed before applying the terms of the Policy.

The following codes are included below for informational purposes. Inclusion or exclusion of a code(s) does not constitute or imply member coverage or provider reimbursement policy. Policy Statements are intended to provide member coverage information and may include the use of some codes for clarity. The Policy Guidelines section may also provide additional information for how to interpret the Policy Statements and to provide coding guidance in some cases.

Type	Code	Description
CPT®	81479	Unlisted molecular pathology procedure
	0306U	Oncology (minimal residual disease [MRD]), next-generation targeted sequencing analysis, cell-free DNA, initial (baseline) assessment to determine a patient-specific panel for future comparisons to evaluate for MRD ***(Code effective 4/1/2022)***
	0307U	Oncology (minimal residual disease [MRD]), next-generation targeted sequencing analysis of a patient-specific panel, cell-free DNA, subsequent assessment with comparison to previously analyzed patient specimens to evaluate for MRD ***(Code effective 4/1/2022)***
	0340U	Oncology (pan-cancer), analysis of minimal residual disease (MRD) from plasma, with assays personalized to each patient based on prior next-generation sequencing of the patient's tumor and germline DNA, reported as absence or presence of MRD, with disease-burden correlation, if appropriate ***(Code effective 10/1/2022)***
HCPCS	None	

Policy History

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

Effective Date	Action
06/01/2022	New policy.

Effective Date	Action
11/01/2022	Coding update
03/01/2023	Annual update. Converted to custom policy. Policy statement, guidelines and literature updated.

Definitions of Decision Determinations

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

Investigational/Experimental: A treatment, procedure, or drug is investigational when it has not been recognized as safe and effective for use in treating the particular condition in accordance with generally accepted professional medical standards. This includes services where approval by the federal or state governmental is required prior to use, but has not yet been granted.

Split Evaluation: Blue Shield of California/Blue Shield of California Life & Health Insurance Company (Blue Shield) policy review can result in a split evaluation, where a treatment, procedure, or drug will be considered to be investigational for certain indications or conditions, but will be deemed safe and effective for other indications or conditions, and therefore potentially medically necessary in those instances.

Prior Authorization Requirements and Feedback (as applicable to your plan)

Within five days before the actual date of service, the provider must confirm with Blue Shield that the member's health plan coverage is still in effect. Blue Shield reserves the right to revoke an authorization prior to services being rendered based on cancellation of the member's eligibility. Final determination of benefits will be made after review of the claim for limitations or exclusions.

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

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

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

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

Appendix A

POLICY STATEMENT	
BEFORE Red font: Verbiage removed	**AFTER** Blue font: Verbiage Changes/Additions
Tumor-Informed Circulating Tumor DNA Testing for Cancer Management 2.04.153 **Policy Statement:** I. Tumor-informed circulating tumor DNA testing (e.g., Signatera) is considered **investigational** for all indications.	**Tumor-Informed Circulating Tumor DNA Testing for Cancer Management** BSC2.18 **Policy Statement:** I. The use of a personalized, tumor-informed circulating tumor DNA (ctDNA) plasma-based test (e.g., Signatera by Natera or Personalized Cancer Monitoring—PCM by Invitae) for solid tumors is considered **medically necessary** when **BOTH** the following are met: A. Individual with stage I-IV cancer after surgical intervention with curative intent to provide information for **any** of the following: 1. Adjuvant or targeted therapy 2. Monitoring for relapse or progression (including but not limited to the use of immunotherapy immune checkpoint inhibitors {e.g., pembrolizumab [Keytruda], ipilimumab [Yervoy], nivilumab [Opdivo]}) B. Frequency of testing does not exceed recommendations for monitoring noted in National Comprehensive Cancer Network (NCCN) guidelines for RECIST (Response Evaluation Criteria in Solid Tumors) for **any** of the following: 1. Initial testing within 4-6 weeks after surgery as a baseline and for adjuvant therapy decisions 2. Every 3-6 months for the first 2 years initially or with recurrence or progression (not to exceed 4 tests/year) 3. Every 6-12 months for the following 3 years (not to exceed 2 tests/year) for colorectal cancer (CRC), NSCLC (Non-Small Cell Lung Cancer) 4. Annually for the following 5 years (not to exceed 1 test/year) 5. As indicated thereafter based on clinicopathologic features II. The use of tumor-informed ctDNA is considered to be **investigational** for individuals with **any** of the following conditions: A. Pregnancy B. Active hematological malignancy C. History of allogeneic bone marrow/stem cell transplants

POLICY STATEMENT	
BEFORE Red font: Verbiage removed	**AFTER** Blue font: Verbiage Changes/Additions
	D. Within 2 weeks after blood transfusion E. Other situations not meeting medically necessary criteria noted above