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Title: Gene Expression Profile Testing and Circulating Tumor DNA Testing for Predicting Recurrence in Colon Cancer

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Populations	Interventions	Comparators	Outcomes
Individuals: • With stage II or III colon cancer	Interventions of interest are: • Gene expression profile testing	Comparators of interest are: • Risk prediction based on clinicopathologic factors	Relevant outcomes include: • Disease-specific survival • Test accuracy • Test validity • Change in disease status
Individuals: • With stage II or III colon cancer	Interventions of interest are: • Circulating tumor DNA testing	Comparators of interest are: • Risk prediction based on clinicopathologic factors	Relevant outcomes include: • Disease-specific survival • Test accuracy • Test validity Change in disease status

DESCRIPTION

Gene expression profiling (GEP) and circulating tumor DNA (ctDNA) tests have been developed for use as prognostic markers in stage II or III colon cancer to help identify patients who are at high-risk for recurrent disease and could be candidates for adjuvant chemotherapy.

OBJECTIVE

The objective of this evidence review is to determine whether gene expression profile testing improves the net health outcome in individuals with stage II or III colon cancer who are being considered for adjuvant chemotherapy.

BACKGROUND

Colon Cancer

According to estimates by the National Cancer Institute, in 202 1 over 147,000 new cases of colorectal cancer will be diagnosed in the U.S., and nearly 53,000 people will die of this cancer.[1,] Five-year survival estimates are around 65%.

Colorectal cancer is classified as stage II (also called Dukes B) when it has spread outside the colon and/or rectum to nearby tissue but is not detectable in lymph nodes (stage III disease, also called Dukes C) and has not metastasized to distant sites (stage IV disease). Primary treatment is surgical resection of the primary cancer and colonic anastomosis. After surgery, the prognosis is good, with survival rates of 75% to 80% at 5 years.[2,] A Cochrane review by Figueredo et al (2008), assessing 50 studies of adjuvant therapy versus surgery alone in stage II patients, found a small though statistically significant absolute benefit of chemotherapy for disease-free survival but not for overall survival. Therefore, adjuvant chemotherapy with 5-fluorouracil or capecitabine is recommended only for resected patients with high-risk stage II disease (ie, those with poor prognostic features).[3,]

However, the clinical and pathologic features used to identify high-risk disease are not well-established, and patients for whom benefits of adjuvant chemotherapy would most likely outweigh harms cannot be identified with certainty. The current diagnostic system relies on a variety of factors, including tumor substage IIB (T4a tumors that invade the muscularis propria and extend into the surface of the visceral peritoneum) or IIC (T4b tumors that invade or are adherent to other organs or structures), obstruction or bowel perforation at initial diagnosis, an inadequately low number of sampled lymph nodes at surgery (< 12), histologic features of aggressiveness, and indeterminate or positive resection margins.[3,]Gene expression profiling and circulating tumor DNA (ctDNA) tests are intended to facilitate identifying stage II patients most likely to experience recurrence after surgery and most likely to benefit from additional treatment.

Of interest, a review by Vilar and Gruber (2010) has noted that microsatellite instability and mismatch repair deficiency in colon cancer may represent confounding factors to be considered in treatment.[4,] These factors may identify a minority (15 to 20%) of the population with improved disease-free survival who may derive no benefit or may exhibit deleterious effects from adjuvant 5-fluorouracil plus leucovorin-based treatments. Patient microsatellite instability and mismatch repair status may be critically important in how to study, interpret, and use a particular gene expression profile test.

REGULATORY STATUS

Clinical laboratories may develop and validate tests in-house and market them as a laboratory service; laboratory-developed tests must meet the general regulatory standards of the Clinical Laboratory Improvement Amendments (CLIA). Multigene expression assay testing and ctDNA testing for predicting recurrent colon cancer is available under the auspices of CLIA. Laboratories that offer laboratory-developed tests must be licensed by the CLIA for high-complexity testing. To date, the U.S. Food and Drug Administration has chosen not to require any regulatory review of this test.

Gene expression profile and ctDNA tests for colon cancer currently commercially available include:

- GeneFx® Colon (Helomics Therapeutics; also known as ColDx, Almac Diagnostics)
- Oncotype DX® Colon Recurrence Score (Genomic Health)
- Signatera™ ctDNA test (Natera)
- Colvera® ctDNA test (Clinical Genomics)

POLICY

A. Gene expression assays for determining the prognosis of stage II or III colon cancer following surgery are considered **experimental / investigational**.

B. Circulating tumor DNA assays for determining the prognosis of stage II or III colon cancer following surgery are considered **experimental / investigational**.

POLICY GUIDELINES

Genetics Nomenclature Update

The Human Genome Variation Society nomenclature is used to report information on variants found in DNA and serves as an international standard in DNA diagnostics. It is being implemented for genetic testing medical evidence review updates starting in 2017 (Table PG1). The Society's nomenclature is recommended by the Human Variome Project, the HUman Genome Organization, and by the Human Genome Variation Society itself.

The American College of Medical Genetics and Genomics and the Association for Molecular Pathology standards and guidelines for interpretation of sequence variants represent expert opinion from both organizations, in addition to the College of American Pathologists. These recommendations primarily apply to genetic tests used in clinical laboratories, including genotyping, single genes, panels, exomes, and genomes. Table PG2 shows the recommended standard terminology—"pathogenic," "likely pathogenic," "uncertain significance," "likely benign," and "benign"—to describe variants identified that cause Mendelian disorders.

Table PG1. Nomenclature to Report on Variants Found in DNA

Previous	Updated	Definition
Mutation	Disease-associated variant	Disease-associated change in the DNA sequence
	Variant	Change in the DNA sequence
	Familial variant	Disease-associated variant identified in a proband for use in subsequent targeted genetic testing in first-degree relatives

Table PG2. ACMG-AMP Standards and Guidelines for Variant Classification

Variant Classification	Definition
Pathogenic	Disease-causing change in the DNA sequence
Likely pathogenic	Likely disease-causing change in the DNA sequence
Variant of uncertain significance	Change in DNA sequence with uncertain effects on disease
Likely benign	Likely benign change in the DNA sequence
Benign	Benign change in the DNA sequence

ACMG: American College of Medical Genetics and Genomics; AMP: Association for Molecular Pathology.

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RATIONALE

This evidence review has been updated regularly with searches of the PubMed database. The most recent literature update was performed through June 14, 2021.

Evidence reviews assess whether a medical test is clinically useful. 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.

The first step in assessing a medical test is to formulate the clinical context and purpose of the test. The test must be technically reliable, clinically valid, and clinically useful for that purpose. Evidence reviews assess the evidence on whether a test is clinically valid and clinically useful. Technical reliability is outside the scope of these reviews, and credible information on technical reliability is available from other sources.

GENE EXPRESSION PROFILE TESTING

Clinical Context and Test Purpose

The purpose of prognostic testing of diagnosed disease is to predict natural disease course (eg, aggressiveness, risk of recurrence, death). This type of testing uses gene expression of affected tissue to predict the course of the disease.

The question addressed in this evidence review is: Does prognostic testing using the gene expression profile (GEP) tests described below in individuals diagnosed with stage II or stage III colon cancer improve the net health outcome?

The specific clinical context of each test is described briefly in the following section. The following PICO was used to select literature to inform this review.

Populations

The relevant population(s) of interest are patients who have undergone surgery for stage II or stage III colon cancer and are being evaluated for adjuvant chemotherapy.

Interventions

The interventions of interest are GEP testing with the GeneFx Colon (ColDx) and Oncotype DX Colon Recurrence Score.

These tests are offered commercially through various manufacturers and would be performed on tumor tissue after surgical resection.

Comparator

The comparator of interest is risk prediction based on clinicopathologic factors. The current standard of care is not to provide adjuvant chemotherapy to patients with stage II colon cancer and to administer adjuvant chemotherapy routinely to patients with stage III colon cancer. However, adjuvant chemotherapy may be considered for patients with stage II colon cancer and poor prognostic features.

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 in patients classified as low-risk, medium-risk, or high-risk by GEP.

The time of interest is 3 to 5 years after surgical resection to assess colon cancer recurrence, given that the majority of colon cancer recurrences occur within the first 3 years after surgical resection of the primary tumor and approximately 95% in the first 5 years.[5,].

Study Selection Criteria

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

- Reported on the accuracy of the marketed version of the technology (including any algorithms used to calculate scores)
- Included a suitable reference standard
- Patient/sample clinical characteristics were described
- Patient/sample selection criteria were described.

Clinically Valid

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

REVIEW OF EVIDENCE

GeneFx Colon

Kennedy et al (2011) reported on the development of a 634-probe set signature.[6,] A training set of 215 patients (142 low-risk, 73 high-risk) was identified based on 5-year disease-free survival. The assay was performed using a DNA-microarray analysis of formalin-fixed, paraffin-embedded (FFPE) samples. Cross-validation studies were used to select an optimal transcript signature for prognostic classification. Independent validation was performed on 144 patients enriched for recurrence (85 low-risk, 59 high-risk) using the threshold score identified in the training set. The signature in this convenience sample of patients predicted disease recurrence with a hazard ratio (HR) of 2.53 ($p<.001$) in the high-risk group. The signature also predicted cancer-related death with an HR of 2.21 ($p<.001$) in the high-risk group.

Niedzwiecki et al (2016) reported on the recurrence-free interval for 393 of 1738 patients treated in the Cancer and Leukemia Group B 9581 (CALGB 9581) trial.[7,] Treatment in CALGB 9581 was with an experimental monoclonal antibody (edrecolomab) or observation; there was no significant survival benefit from the experimental treatment. Of 901 eligible patients with available tissue, a randomized sample of 514 patients was selected. The final analysis included 360 patients in the randomized cohort (58 events) and 33 nonrandomly selected events that had samples successfully analyzed. The investigators hypothesized that the high failure rate was due to the long interval between sample collection and analysis (mean, 13.2 years). Table 1 provides recurrence scores for patients categorized as low-risk and high-risk. After adjusting for prognostic variables that included mismatch repair deficiency, patients categorized as high-risk by GeneFx had a significantly worse recurrence-free interval in unadjusted analysis (HR, 2.13; 95%

confidence interval [CI], 1.3 to 3.5; p<.01). However, in multivariate analysis, the GeneFx risk score was marginally associated with overall survival (HR, 1.74; 95% CI, 0.97 to 3.1; p=.06). For the 271 samples analyzed by both GeneFx and Oncotype DX (see below), there was a weak correlation in continuous scores (*R* = 0.18).

Table 1. Recurrence-free Survival in Patients With Stage II Colon Cancer Assessed With GeneFx

Study	N	Follow-Up, y	Low Risk, n (%)	Mean RFS for Low Risk (95% CI)	High Risk, n (%)	Mean RFS for High Risk (95% CI)
Niedzwecki et al (2016)[7,]	393	5	177 (45)	91 (89 to 93)	216 (55)	82 (79 to 85)

CI: confidence interval; RFS: recurrence-free survival; y: years.

Oncotype DX Colon Recurrence Score

O'Connell et al (2010) described the development of a 12-gene expression test called Oncotype DX Colon Recurrence Score.[8,] A total of 761 candidate genes of possible prognostic value for recurrence or of possible predictive value for treatment were examined by correlating the genes in tumor samples with clinical outcomes in 1851 patients who had surgery with or without adjuvant 5-fluorouracil-based chemotherapy. Gene expression was quantified from microdissected, FFPE primary colon cancer tissue. Of the 761 candidate genes, multivariate analysis (including disease severity, stage, and nodal involvement) reduced the gene set to a 7-gene prognostic signature and a separate 6-gene predictive signature. Five reference genes also are included in the assay.

Tables 2 and 3 summarize the characteristics and results of several validation studies. External validation of the algorithm was first reported by Gray et al (2011), who used FFPE primary tumor samples from patients with stage II colon cancer who had participated in the Quick and Simple and Reliable (QUASAR) study.[9,] The relation between the 7-gene recurrence score and risk of recurrence was statistically significant, with a 3-year risk of recurrence for predefined low-, intermediate-, and high-risk groups as shown in Table 3. In the surgery-alone group, the HR for recurrence in the high-risk group compared with the low-risk group was 1.47 (95% CI, 1.01 to 2.14, p=.046).

Table 2. Oncotype DX Colon Validation Study Characteristics

Study; Trial	Design	N	Colon Cancer, n		Randomized Treatments	
			Stage II	Stage III	Intervention	Comparator
Gray et al (2011)[9,]; QUASAR	RCT	3239	1436		Adjuvant chemotherapy	Surgery alone
Venook et al (2013)[10,]; CALGB 9581	RCT	1713	690		Edrecolomab	Observation

Study; Trial	Design	N	Colon Cancer, n		Randomized Treatments	
Yothers et al (2013)[11,]; NASBP C-07 R	RCT	2409	264		5-fluorouracil plus leucovorin with oxaliplatin	5-fluorouracil plus leucovorin without oxaliplatin
Reimers et al (2014)[12,]; TME	RCT	1861	130[a]	167[a]	Radiotherapy	No radiotherapy
Yamanaka et al (2016)[13,]; SUNRISE	Cohort	1487	247	350	Not applicable	

CALGB 9581: Cancer and Leukemia Group B 9581 trial; NASBP C-07: National Surgical Adjuvant Breast and Bowel Project; QUASAR: Quick and Simple and Reliable; RCT: randomized controlled trial; TME: Dutch total mesenteric excision trial.

[a] Rectal.

Venook et al (2013) reported on a validation study using tumor tissue from patients with stage II colon cancer who had participated in the randomized CALGB 9581 trial.[10,] The investigators selected samples stratified by treatment group from those who had tumor tissue available (40% of the original patient sample). They used recurrence score cut points of 29 and 39 to determine low-, intermediate-, and high-risk groups (Table 3); these values differ from the cut points of 30 and 41 validated in the QUASAR study (previously described). In multivariate analysis, every 25-unit change in recurrence score was associated with recurrence independent of tumor stage, tumor grade, mismatch repair status, presence or absence of lymphovascular invasion, and the number of nodes assessed.

Yothers et al (2013) conducted a validation study using tumor tissue from 264 patients with stage II colon cancer who had participated in the National Surgical Adjuvant Breast and Bowel Project C-07 (NASBP C-07) trial.[11,] The NASBP C-07 trial randomized 2409 patients with stage II (28%) or stage III (72%) colon cancer to adjuvant chemotherapy with 5-fluorouracil plus leucovorin or oxaliplatin plus 5-fluorouracil plus leucovorin. For the randomly selected sample of 50% of patients with stage II colon cancer, estimated 5-year recurrence risks (adjusted for treatment) are shown in Table 3. Five-year recurrence risk, estimated by Kaplan-Meier analysis, was reduced in high-risk patients who received oxaliplatin (9%; 95% CI, 3% to 25%) compared with those who did not (23%; 95% CI, 12% to 42%) but this difference was not observed in low- or intermediate-risk patients. However, CIs for these estimates were wide due to small numbers of patients and events in each risk group. For all stage III patients in any risk class, adjusted 5-year recurrence risk estimates exceeded 15%.

Table 3. Recurrence Rates by Risk Category for the Oncotype DX Colon Recurrence Risk Score

Study	Trial	Risk Prediction, y	Mean Recurrence Rate (95% CI), %		
			Low Risk	Medium Risk	High Risk
Gray et al (2011)[9,]	QUASAR	3	12	18	22

Study	Trial	Risk Prediction, y	Mean Recurrence Rate (95% CI), %		
Venook et al (2013)[10,]	CALGB 9581	5	12 (10 to 15)	15 (12 to 17)	18 (14 to 22)
Yothers et al (2013)[11,]	NASBP C-07	5	9 (6 to 13)	13 (8 to 17)	18 (12 to 25)
Reimers et al (2014)[12,]	TME stage II cohort (rectal)	5	11 (6 to 22)	27 (16 to 46)	43 (29 to 65)
Yamanaka et al (2016)[13,]	SUNRISE stage II cohort	5	9 (7 to 12)	14 (11 to 17)	19 (13 to 24)
	SUNRISE stage III cohort	5	20 (14 to 25)	29 (23 to 35)	38 (29 to 47)

CALGB 9581: Cancer and Leukemia Group B 9581 trial; CI: confidence interval; NASBP C-07: National Surgical Adjuvant Breast and Bowel Project; QUASAR: Quick and Simple and Reliable; TME: Dutch total mesenteric excision trial; y: years.

Reimers et al (2014)[12,] conducted a retrospective study using prospectively collected tumor specimens from the Dutch total mesenteric excision trial[14,] in patients with resectable rectal cancer. Reimers et al (2014) used available tumor tissue from 569 stage II and III patients randomized to surgery alone.[12,] Among 130 patients with stage II rectal cancer, Oncotype DX Colon classified 63 (49%) patients as low-risk, 37 (28%) patients as intermediate-risk, and 30 (23%) patients as high-risk. Five-year Kaplan-Meier recurrence risk estimates in the low-, intermediate-, and high-risk groups are shown in Table 3. Oncotype DX Colon risk classification and estimated recurrence risks for patients with stage III rectal cancer were not reported.

The SUNRISE study, as reported by Yamanaka et al (2016), evaluated tissue samples from consecutive patients with stage II and stage III colon cancer who had been treated with surgery alone.[13,] Surgery was the standard of care at hospitals in Japan during the study period 2000 to 2005. From the total cohort of 1487 patients, samples were randomly selected from patients who had or did not have a recurrence, in a 1:2 ratio. The final number of patients studied was 597; 202 patients had disease recurrence and 395 had no recurrence. As shown in Table 3, the risk of recurrence in patients with stage III colon cancer with a low-risk score was similar to patients with stage II disease and a high-risk score and exceeded 15%. When adjusted for disease stage, a 25-unit increase in the recurrence score had an HR of 2.05 (95% CI, 1.47 to 2.86; p<.001).

Section Summary: Clinically Valid

Several validation studies of GEP testing for colon cancer have reported that testing provides prognostic information on the risk of recurrence. Some studies have reported that GEP testing offers prognostic information in a multivariate analysis. Patients with a low recurrence score have a lower risk of recurrence and patients with a high-risk score have a higher risk of recurrence. However, the increase in recurrence risk for a high-risk score is small, and it is uncertain whether the degree of increase is sufficient to intensify management.

Clinically Useful

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

REVIEW OF EVIDENCE

Direct Evidence

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

A technical brief by Black et al (2012), conducted for the Agency for Healthcare Research and Quality, reviewed the clinical evidence for GEP testing in predicting outcomes, including the benefit from adjuvant chemotherapy, in patients with stage II colon cancer.[15,] The 2 commercially available assays reviewed herein were included in the brief. No prospective studies were identified that assessed change in the net health outcome with the use of a GEP assay, and no studies were identified that used a net reclassification analysis and subsequently evaluated the impact of the reclassification on the net health outcome. Additionally, evidence was limited on the reproducibility of test findings, indications for GEP testing in stage II patients, and whether results of GEP assays can stratify patients into groups with clinically meaningful differences in recurrence risk. No studies have been identified in subsequent literature updates that evaluated the impact of GEP testing on recurrence in patients with stage II or III colon cancer.

A more recent evidence report conducted for the Washington State Health Care Authority (2017) reviewed the clinical utility of GEP tests for cancer, including Oncotype DX for stage II or III colon cancer.[16,] The researchers identified no clinical utility studies with mortality, morbidity, or harms outcomes.

Chain of Evidence

Indirect evidence on clinical utility rests on clinical validity. If the evidence is insufficient to demonstrate test performance, no inferences can be made about clinical utility. A chain of evidence may be developed, which addresses 2 key questions.

1. Does the use of GEP testing of colon cancer risk in individuals with stage II or stage III colon cancer lead to a change in management regarding the use of adjuvant chemotherapy?
2. Do those management changes improve health outcomes?

Several studies have documented changes in management following GEP testing with the Oncotype DX Colon Cancer Assay. For example, Oki et al (2021) published a prospective observational study in Japan examining the impact of Oncotype Dx Colon Recurrence Score on management decisions for patients with stage II and stage IIIA/IIIB colon cancer.[17,] The study included 275 patients; 97 patients had stage II colon cancer, and 178 had stage IIIA/IIIB disease. Oncotype Dx Colon Recurrence Score changed treatment decisions in 39.6% of patients. Treatment was decreased in intensity in 32% of study patients (n=88), and increased in intensity for 7.6% of study patients (n=21). Patients with stage IIIA/IIIB cancer had treatment recommendations changed more frequently than patients with stage II cancer (44.9% vs.

29.9%; p=.0148). Similarly, Brenner et al (2016) published a retrospective study of the association between Oncotype DX Colon Recurrence Score and management decisions.[18,] The study included 269 patients from a health plan who had stage II colon cancer, mismatch repair proficient status, and Oncotype DX Colon Recurrence Score. The primary outcome measure was change in management that occurred following Oncotype DX Colon testing. Patients were classified as having either an increase in the intensity of surveillance or treatment, a decrease in the intensity of surveillance or treatment, or no change. A change in management following testing was found for 102 (38%) of 269 patients. Of the 102 patients with management changes, 76 patients had a decrease and 26 had an increase in treatment intensity. More patients who had a low recurrence score had a decrease in the intensity of management, and more patients with a high recurrence score had an increase in intensity.

Cartwright et al (2014) [19,] and Srivastava et al (2014) [20,] have also published studies showing the effect of Oncotype DX Colon results on treatment recommendations made using traditional risk classifiers in patients with stage II colon cancer. Cartwright et al (2014) performed a retrospective study predicting that test results might lead to reductions in treatment intensity in a percentage of patients.[19,] Srivastava et al (2014) performed a prospective study that directly demonstrated reductions in treatment intensity in a percentage of patients.[20,]

This type of study does not determine whether patient outcomes are improved as a consequence of the changes in management, and there are no well-defined treatment protocols that differ according to the risk of recurrence within stage II or within stage III colon cancer.

Section Summary: Clinically Useful
Some studies have reported management changes following GEP testing. However, these studies did not report clinical outcomes, and there is no direct evidence to determine whether GEP testing improves health outcomes. A chain of evidence might be constructed if there was evidence that changes in management for patients with stage II or III colon cancer improved health outcomes. The intensity of surveillance and management may be impacted by results of GEP testing but the evidence to demonstrate that a change in management improved health outcomes is weak and not definitive. Therefore, the evidence does not demonstrate clinical utility.

CIRCULATING TUMOR DNA TESTING

Clinical Context and Test Purpose
The purpose of prognostic testing of diagnosed disease is to predict natural disease course (eg, aggressiveness, risk of recurrence, death). This type of testing uses circulating tumor DNA (ctDNA) testing of blood to predict the course of the disease.

The question addressed in this evidence review is: Does prognostic testing using the ctDNA tests described below in individuals diagnosed with stage II or stage III colon cancer improve the net health outcome?

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

Populations

The relevant populations of interest are patients with stage II or III colon cancer who have undergone surgery and are being evaluated for adjuvant chemotherapy and patients who are being monitored for risk of relapse following treatment for stage II or III colon cancer.

Interventions

The intervention of interest is ctDNA testing with the Signatera or Colvera assays. Signatera is designed to detect molecular residual disease in the blood. Tumor tissue obtained from either a diagnostic biopsy or surgically resected tissue is used to identify 16 single nucleotide variants found in the tumor but not in normal tissue. Once the tumor has been definitively treated, a custom assay of 16 tumor-specific clonal, somatic variants is generated for the patient and the resulting tumor signature is monitored throughout the patient's disease course. The Colvera assay is designed to detect 2 methylated genes that are associated with colorectal tumor tissue, BCAT1 and IKZF1, in ctDNA in the blood.

Comparator

The comparator of interest is risk prediction based on clinicopathologic factors. For patients with stage II colon 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 patients with stage II colon cancer, using clinicopathologic characteristics to identify patients who might benefit.[3,] For patients with stage III colon cancer, the current standard of care is to administer adjuvant chemotherapy routinely. For patients who are being monitored for risk of relapse following treatment for stage II or III colon 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.

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.

Given that the majority of colon 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.[5,].

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

Study Selection Criteria

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

- Reported on the accuracy of the marketed version of the technology (including any algorithms used to calculate scores)
- Included a suitable reference standard
- Patient/sample clinical characteristics were described
- Patient/sample selection criteria were described.

Clinically Valid

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

REVIEW OF EVIDENCE

Signatera Assay

A cohort study that used the Signatera assay reported an association between positive ctDNA results and risk of recurrence of colon cancer (Tables 4 and 5).[21,] Study limitations are described in Tables 6 and 7.

Reinert et al (2019) enrolled 125 patients with stage I to III colon cancer in a validation study of the Signatera assay.[21,] 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 patients 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.

Table 4. Signatera ctDNA Assay Study Characteristics

Study	Design	Detection Method	N	Data Collection	Colon Cancer, n		
					Stage I	*Stage II*	*Stage III*
Reinert et al (2019)[21,]	Cohort	Signatera Assay	125	Day 30 following surgery, up to 3 years	5	39	81

ctDNA: circulating tumor DNA.

Table 5. Recurrence Rates by Risk Category for Signatera ctDNA Assay

Study	Mean Recurrence Rate (95% CI)	
	ctDNA Positive	**ctDNA Negative**
Reinert et al (2019)[21,]	7/10; 70% (34.2% to 93.1%)	10/84; 11.9% (6.3% to 20.1%)
Hazard ratio for RFS (95% CI)	7.2 (2.7 to 19.0); p<.001	

CI: confidence interval; ctDNA: circulating tumor DNA; RFS: recurrence-free survival.

Table 6. Study Relevance Limitations

Study	Population[a]	Intervention[b]	Comparator[c]	Outcomes[d]	Duration of Follow-Up[e]
Reinert et al (2019)[21,]	1. Included patients with stage I through III colon cancer		3. No comparator	1. Overall survival 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 7. 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)[21,]	1. Patient selection not described					Multiple subgroup analyses, small numbers of patients with positive ctDNA tests.

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.

Colvera Assay

Three cohort studies have reported an association between positive ctDNA results and risk of recurrence of colon cancer (Tables 8 and 9).[22,23,24,] Limitations of these studies are described in Tables 10 and 11.

Young et al (2016) enrolled 122 patients with colorectal cancer who had no evidence of residual disease after initial therapy.[22,] In this study, a positive ctDNA test was associated with an increased risk of recurrence. Blood samples were also tested for CEA, and a positive CEA test was also found to be significantly associated with an increased risk of recurrence. Among the 28 patients who had recurrent disease, 9 patients (32%) had a positive CEA test, while 19 (68%) had a positive ctDNA test (p=.002). Among the 94 patients without clinically detectable recurrence, CEA was positive in 6 patients (6%) and ctDNA test was positive in 12 (13%; p=.210). The positive predictive values of ctDNA and CEA were 61.3% and 60%, respectively. The negative predictive values were 90.1% and 82.2%, respectively.

Murray et al (2018) enrolled 172 patients with invasive colorectal cancer with plasma samples collected within 12 months after surgery.[23,] In this study, multivariate analysis found that risk of recurrence was increased among patients who had positive ctDNA tests following surgery. Risk of colorectal cancer-related death was also increased among patients who had a positive ctDNA test following surgery, but multivariate analysis could not be performed for this outcome due to the low number of events.

Symonds et al (2020) examined the association between a positive Colvera test result and recurrence of colorectal cancer in 144 patients who had no evidence of residual disease after surgical resection and/or neoadjuvant chemotherapy.[24,] Blood samples were also tested for CEA, and the association between a positive CEA test and recurrent colorectal cancer was assessed. A positive Colvera test was an independent predictor of recurrence, while a positive CEA test was not found to be a significant predictor of recurrence after adjusting for other predictors of recurrence (eg, stage at primary diagnosis). Sensitivity of the Colvera assay for detecting recurrence was significantly greater than the sensitivity of CEA (66% vs. 31.9%, p=.001), but specificity was not significantly different (97.9% vs. 96.4%, p=1.000). The positive predictive value was not significantly different for Colvera and CEA (94.3% vs. 83.3%, p=.262), but the negative predictive value was significantly greater for Colvera (84.4% vs. 71.7%, p<.001).

Musher et al (2020) conducted an additional prospective cross-sectional observational study in patients undergoing surveillance after definitive therapy for stage II or III colorectal cancer.[25,] Samples were collected within 6 months of planned radiologic surveillance imaging and tested using the Colvera assay and a CEA assay. A total of 322 patients were included, with 27 experiencing recurrence and 295 not experiencing recurrence. The sensitivities of Colvera and CEA for detecting colorectal cancer recurrence using a single time-point blood test were 63% (17/27) and 48.1% (13/27), respectively (p=.046). The specificities of single time-point Colvera and CEA were 91.5% and 96.3%, respectively (p=.012).

Table 8. Colvera Assay Observational Study Characteristics

Study	Design	Detection Method	Comparator Test	N	Data Collection	Colon Cancer, n			
						Stage I	*Stage II*	*Stage III*	*Stage IV*
Young et al (2016)[22,]	Cross-sectional observational	Colvera assay	CEA	122[a]	Sample collected 12 months prior	28	40	47	6

Study	Design	Detection Method	Comparator Test	N	Data Collection	Colon Cancer, n			
					to or 3 months after complete investigational assessment of recurrence status				
Murray et al (2018)[23,]	Prospective cohort	Colvera assay	None	172	Single sample collected within 12 months of surgical resection	NR	NR	NR	NR
Symonds et al (2020)[24,]	Cross-sectional observational	Colvera assay	CEA	144	Single sample collected at time of recurrence or within 12 months of surveillance imaging	21	50	62	11

CEA: carcinoembryonic antigen; ctDNA: circulating tumor DNA; NR: not reported.
[a]1 patient in this study had unstaged primary cancer.

Table 9. Recurrence Rates by Risk Category for Colvera Assay

Study	Recurrence Rate (95% CI)	
Young et al (2016)[22,]	28/122	
Positive vs. negative Colvera odds ratio for recurrence (95% CI)	14.4 (5.4 to 38.7; p<.001)	
Positive vs. negative CEA odds ratio for recurrence (95% CI)	6.9 (2.3 to 21.1; p=.001)	
	ctDNA Positive	*ctDNA Negative*
Murray et al (2018)[23,]	7/28	16/144
Positive vs. negative Colvera hazard ratio for recurrence (95% CI)	3.8 (1.5 to 9.5; p=.004)	
Positive vs. negative Colvera hazard ratio for colorectal cancer-related death (95% CI)	6.6 (1.9 to 22.8)	
Symonds et al (2020)[24,]	50/144	
Positive vs. negative Colvera adjusted odds ratio for recurrence (95% CI)	155.7 (17.9 to 1360.6; p<.001)	
Positive vs. negative CEA adjusted odds ratio for recurrence (95% CI)	2.5 (0.3 to 20.6; p=.407)	

CEA: carcinoembryonic antigen; CI: confidence interval; ctDNA: circulating tumor DNA.

Table 10. Study Relevance Limitations

Study	Population[a]	Intervention[b]	Comparator[c]	Outcomes[d]	Duration of Follow-Up[e]
Young et al (2016)[22,]	1. Included patients with any stage of colon cancer			1. Overall survival not assessed	
Murray et al (2018)[23,]	1. Included patients with any stage of colon cancer		3. No comparator		
Symonds et al (2020)[24,]	1. Included patients with any stage of colon cancer			1. Overall survival 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 11. Study Design and Conduct Limitations

Study	Selection[a]	Blinding[b]	Delivery of Test[c]	Selective Reporting[d]	Data Completeness[e]	Statistical[f]
Young et al (2016)[22,]						
Murray et al (2018)[23,]	1. Patient selection not described		1. Timing of sample collection could be any time within 12 months following surgery			2. Not compared to other tests

Study	Selection[a]	Blinding[b]	Delivery of Test[c]	Selective Reporting[d]	Data Completeness[e]	Statistical[f]
Symonds et al (2020)[24,]	1. Patient selection not described					

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.

Clinically Useful
A test is clinically useful if the use of the results informs management decisions that improve the net health outcome of care. The net health outcome can be improved if patients receive correct therapy, more effective therapy, or avoid unnecessary therapy or testing. No studies of the clinical utility of ctDNA were identified.

REVIEW OF EVIDENCE

Direct Evidence
Direct evidence of clinical utility is provided by studies that have compared health outcomes for patients managed with and without the test. Because these are intervention studies, the preferred evidence would be from randomized controlled trials. There is no direct evidence of the clinical utility of ctDNA testing in patients with colon cancer.

Chain of Evidence
Indirect evidence on clinical utility rests on clinical validity. If the evidence is insufficient to demonstrate test performance, no inferences can be made about clinical utility. A chain of evidence may be developed, which addresses 2 key questions.

1. Does the use of ctDNA testing of colon cancer risk in individuals with stage II or stage III colon cancer lead to a change in management regarding the use of adjuvant chemotherapy?
2. Do those management changes improve health outcomes?

In observational studies of the association of ctDNA to risk of recurrence in colon cancer, management decisions were not based on ctDNA test results.

Section Summary
Several observational studies reported an association between positive ctDNA results using the Signatera assay or Colvera assay and risk of recurrence of colon cancer. While these studies showed an association between ctDNA results and risk of recurrence, they are limited by their observational design and relatively small numbers of patients. Management decisions were not based on ctDNA test results. There are no controlled studies of management changes made in

response to ctDNA test results compared to other risk factors, and no studies showing whether testing improved outcomes.

Summary of Evidence
For individuals who have stage II or III colon cancer who receive GEP testing, the evidence includes development and validation studies and decision-impact studies. Relevant outcomes are disease-specific survival, test accuracy and validity, and change in disease status. The available evidence has shown that GEP testing for colon cancer can improve risk prediction, particularly the risk of recurrence in patients with stage II or III colon cancer. However, the degree of difference in risk conferred by the test is small. Evidence to date does not permit conclusions on whether GEP classification is sufficient to modify treatment decisions in stage II or III patients. Studies showing management changes as a consequence of testing have not demonstrated whether such changes improve outcomes. The evidence is insufficient to determine that the technology results in an improvement in the net health outcome.

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

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
Current clinical practice guidelines from the National Comprehensive Cancer Network (v. 2.2021) on colon cancer state that "there are insufficient data to recommend the use of multigene assays...or post-surgical ctDNA to estimate risk of recurrence or determine adjuvant therapy" in patients with stage II or III colon cancer.[3,]

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

Ongoing and Unpublished Clinical Trials

Some ongoing trials that might influence this review are listed in Table 12.

Table 12. Summary of Key Trials

NCT No.	Trial Name	Planned Enrollment	Completion Date
Ongoing			
NCT04264702[a]	BESPOKE Study of ctDNA Guided Therapy in Colorectal Cancer	1,000	Jun 2024

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

CODING

The following codes for treatment and procedures applicable to this policy are included below for informational purposes. This may not be a comprehensive list of procedure codes applicable to this policy.

Inclusion or exclusion of a procedure, diagnosis or device code(s) does not constitute or imply member coverage or provider reimbursement. 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.

The code(s) listed below are medically necessary ONLY if the procedure is performed according to the "Policy" section of this document.

CPT/HCPCS

81525 Oncology (colon), mRNA, gene expression profiling by real-time RT-PCR of 12 genes (7 content and 5 housekeeping), utilizing formalin-fixed paraffin-embedded tissue, algorithm reported as a recurrence score

ICD-10 DIAGNOSES

Experimental / Investigational for all diagnoses related to this medical policy.

REVISIONS

03-04-2016	Policy added to the bcbsks.com web site on 02-03-2016.
10-12-2016	Updated Description section.
	Updated Rationale section.
	In Coding section: ▪ Revised coding bullets.
	Updated References section.
09-28-2017	Updated Description section.
	In Policy section: ▪ Removed Policy Guidelines.
	Updated Rationale section.
	In Coding section: ▪ Updated Coding bullets.
	Updated References section.
10-01-2018	Updated Description section.
	In Policy section: ▪ Added Policy Guidelines.
	Updated Rationale section.
	Updated References section.
	Removed Appendix section.
03-10-2021	Changed the title from "Multigene Expression Assay for Predicting Recurrence in Colon Cancer" to "Gene Expression Profile Testing and Circulating Tumor DNA Testing for Predicting Recurrence in Colon Cancer"
	Updated Description section.
	In Policy section • Item A- Removed "stage"

	• Added Item B: "Circulating tumor DNA assays for determining the prognosis of stage II or III colon cancer following surgery are considered **experimental / investigational**."
	Updated Rationale section.
	Updated coding section: • Removed CPT codes 81599, 84999, 88299
	Updated References section.
12-02-2021	Updated Description Section
	Updated Rationale Section
	Updated References Section

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