Medical Policy

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Title: Biomarker Testing (Including Liquid Biopsy) for Targeted Treatment and Immunotherapy in Metastatic Colorectal Cancer

Related Policies:	▪ *Genetic Testing for Lynch Syndrome and Other Inherited Colon Cancer Syndromes* ▪ *Comprehensive Genomic Profiling for Selecting Targeted Cancer Therapies* ▪ *Gene Expression Profile Testing and Circulating Tumor DNA Testing for Predicting Recurrence in Colon Cancer* ▪ *Circulating Tumor DNA and Circulating Tumor Cells for Cancer Management (Liquid Biopsy)*

Professional
Original Effective Date: July 10, 2015
Revision Date(s): July 10, 2015; January 1, 2016; August 29, 2016; January 30, 2018; August 29, 2018; September 27, 2019; October 1, 2019; April 30, 2021; October 10, 2021
Current Effective Date: October 10, 2021

Institutional
Original Effective Date: July 10, 2015
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Current Effective Date: October 10, 2021

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Populations	Interventions	Comparators	Outcomes
Individuals: • With metastatic colorectal cancer	Interventions of interest are: • *KRAS* variant testing to guide treatment	Comparators of interest are: • No *KRAS* variant testing to guide treatment	Relevant outcomes include: • Overall survival • Disease-specific survival • Change in disease status • Medication use • Resource utilization • Treatment-related morbidity
Individuals: • With metastatic colorectal cancer	Interventions of interest are: • *NRAS* variant testing to guide treatment	Comparators of interest are: • No *NRAS* variant testing to guide treatment	Relevant outcomes include: • Overall survival • Disease-specific survival • Change in disease status • Medication use • Resource utilization • Treatment-related morbidity
Individuals: • With metastatic colorectal cancer	Interventions of interest are: • *BRAF* variant testing to guide treatment	Comparators of interest are: • No *BRAF* variant testing to guide treatment	Relevant outcomes include: • Overall survival • Disease-specific survival • Change in disease status • Medication use • Resource utilization • Treatment-related morbidity
Individuals: • With metastatic colorectal cancer	Interventions of interest are: • *MMR/MSI* testing to guide treatment	Comparators of interest are: • No MMR/MSI variant testing to guide treatment	Relevant outcomes include: • Overall survival • Disease-specific survival • Change in disease status • Medication use • Resource utilization • Treatment-related morbidity
Individuals: • With metastatic colorectal cancer	Interventions of interest are: • *HER2* testing to guide treatment	Comparators of interest are: • No HER2 variant testing to guide treatment	Relevant outcomes include: • Overall survival • Disease-specific survival • Change in disease status • Medication use • Resource utilization • Treatment-related morbidity
Individuals: • With metastatic colorectal cancer	Interventions of interest are: • Tumor mutational burden testing to guide treatment	Comparators of interest are: • No tumor mutational burden testing to guide treatment	Relevant outcomes include: • Overall survival • Disease-specific survival • Change in disease status • Medication use • Resource utilization • Treatment-related morbidity
Individuals: • With metastatic colorectal cancer	Interventions of interest are: • Testing of circulating tumor DNA to select treatment	Comparators of interest are: • Using tissue biopsy to guide treatment	Relevant outcomes include: • Overall survival • Disease-specific survival • Test validity • Morbid events • Medication use

DESCRIPTION

The epidermal growth factor receptor (EGFR) is overexpressed in colorectal cancer (CRC). EGFR-targeted therapy combined with monoclonal antibodies cetuximab and panitumumab has shown a clear survival benefit in patients with metastatic CRC. However, this benefit depends on a lack of variants in certain genes in the signaling pathway downstream from the EGFR. It has been hypothesized that knowledge of tumor cell *KRAS, NRAS*, and *BRAF* variant status might be used to predict nonresponse to anti-EGFR monoclonal antibody therapy. More recently, testing for

microsatellite instability/mismatch repair (MSI/MMR) and tumor mutational burden (TMB) status to select patients for immunotherapy, and human epidermal growth factor receptor 2 (HER2) testing to select patients for targeted therapy, has been proposed. Typically, the evaluation of biomarker status requires tissue biopsy. Circulating tumor DNA or circulating tumor cell testing (also known as liquid biopsy) is proposed as a non-invasive alternative.

Objective

The objective of this evidence review is to determine whether using biomarker testing to select targeted treatment and immunotherapy improves the net health outcome in individuals with metastatic colorectal cancer.

Background

KRAS, *NRAS*, and *BRAF* Variants

Cetuximab (Erbitux®; ImClone Systems) and panitumumab (Vectibix®; Amgen) are monoclonal antibodies that bind to the epidermal growth factor receptor (EGFR), preventing intrinsic ligand binding and activation of downstream signaling pathways vital for cancer cell proliferation, invasion, metastasis, and stimulation of neovascularization. The RAS-RAF-MAP kinase pathway is activated in the EGFR cascade. The RAS proteins are G proteins that cycle between active (RAS guanosine triphosphate) and inactive (RAS guanosine diphosphate) forms in response to stimulation from a cell surface receptor, such as EGFR, and they act as a binary switch between the cell surface EGFR and downstream signaling pathways. The *KRAS* gene can harbor oncogenic variants that result in a constitutively activated protein, independent of EGFR ligand binding, rendering antibodies to the upstream EGFR ineffective. Approximately 40% of colorectal cancers (CRCs) have *KRAS* variants in codons 12 and 13 in exon 2. Another proto-oncogene that acts downstream from *KRAS*-*NRAS* harbors oncogenic variants in codons 12, 13, or 61 that result in constitutive activation of the EGFR-mediated pathway. These variants are less common compared with *KRAS,* detected in 2% to 7% of CRC specimens. It is unclear whether *NRAS* variants predict poor response due to anti-EGFR monoclonal antibody therapy or are prognostic of poor CRC outcomes in general. A third proto-oncogene, *BRAF*, encodes a protein kinase and is involved in intracellular signaling and cell growth; *BRAF* is also a principal downstream effector of *KRAS*. *BRAF* variants occur in fewer than 10% to 15% of CRCs and appear to be a marker of poor prognosis. *KRAS* and *BRAF* variants are considered to be mutually exclusive.

Cetuximab and panitumumab have marketing approval from the U.S. Food and Drug Administration (FDA) for the treatment of metastatic CRC in the refractory disease setting. The FDA approval for panitumumab indicates that panitumumab is not indicated for the treatment of patients with *KRAS* or *NRAS* variant-positive disease in combination with oxaliplatin-based chemotherapy.[1,]

A large body of literature has shown that metastatic CRC tumors with a variant in exon 2 (codon 12 or 13) of the *KRAS* gene do not respond to cetuximab or panitumumab therapy. More recent evidence has shown that variants in *KRAS* outside exon 2 (ie, in exons 3 [codons 59 and 61] and exon 4 [codons 117 and 146]) and variants in *NRAS* exon 2 (codons 12 and 13), exon 3 (codons 59 and 61), and exon 4 (codons 117 and 146) also predict a lack of response to these monoclonal antibodies. Variant testing of these exons outside the *KRAS* exon 2 is referred to as extended *RAS* testing.

Human Epidermal Growth Factor Receptor 2 Amplification/Overexpression

Human epidermal growth factor receptor 2 (HER2) is a member of the HER (EGFR) family of tyrosine kinase receptors and has no specific ligand. When activated, it forms dimers with other EGFR family members. Amplification of HER2 is detected in approximately 3% of patients with CRC, with higher prevalence in *RAS/BRAF*-wild type tumors (5% to 14%). In addition to its role as a predictive marker for HER2-targeted therapy, HER2 amplification/overexpression is being investigated as a predictor of resistance to EGFR-targeting monoclonal antibodies.

Mismatch Repair Deficiency/Microsatellite Instability
Mismatch repair deficiency (dMMR) and high levels of microsatellite instability (MSI-H) describe cells that have alterations in certain genes involved in correcting errors made when DNA is replicated. Tumors with dMMR are characterized by a high tumor mutational load and potential responsiveness to anti-PD-L1-immunotherapy. Deficiency in MMR is most common in CRC, other types of gastrointestinal cancer, and endometrial cancer, but it may also be found in other cancers including breast cancer. Testing of MSI is generally performed using polymerase chain reaction (PCR) for 5 biomarkers, although other biomarker panels and next generation sequencing are sometimes performed. High MSI is defined as 2 or more of the 5 biomarkers showing instability or more than 30% of the tested biomarkers showing instability depending on what panel is used. Microsatellite instability testing is generally paired with immunohistochemistry assessing lack of protein expression from 4 DNA mismatch repair genes thereby reflecting dMMR.

Tumor Mutational Burden
Tumor mutational burden (TMB), a measure of gene mutations within cancer cells, is an emerging biomarker of outcomes with immunotherapy in multiple tumor types. Initially, assessments of TMB involved whole exome sequencing. More recently, targeted next generation sequencing panels are being adapted to estimate TMB. Currently FoundationOne CDx is the only U.S. Food and Drug Administration (FDA) approved panel for estimating TMB, but others are in development.

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

Typically, the evaluation of RAS mutation status requires tissue biopsy. Circulating tumor DNA (ctDNA) testing is proposed as a non-invasive alternative.

Detection of ctDNA is challenging because ctDNA is diluted by nonmalignant circulating DNA and usually represents a small fraction (<1%) of total ctDNA. Therefore, more sensitive methods than the standard sequencing approaches (eg, Sanger sequencing) are needed.

Highly sensitive and specific methods have been developed to detect ctDNA, for both single nucleotide variants (eg BEAMing [which combines emulsion polymerase chain reaction with magnetic beads and flow cytometry] and digital polymerase chain reaction) and copy-number

variants. Digital genomic technologies allow for enumeration of rare variants in complex mixtures of DNA.

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

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

A number of liquid biopsy tests related to targeted treatment of metastatic colorectal cancer have been developed (Table 1).

Table 1. Examples of Liquid Biopsy Tests Related to Targeted Treatment of Metastatic Colorectal Cancer

Manufacturer	Test	Type of Liquid Biopsy
Biocept	Target Selector™ ctDNA EGFR Kit	ctDNA
Foundation Medicine	FoundationOne Liquid (Previously FoundationAct)	ctDNA
Guardant Health	Guardant360®	ctDNA
IV Diagnostics	Velox™	CTC
Personal Genome Diagnostics	PlasmaSELECT™	ctDNA
Sysmex Inostics	OncoBEAM	ctDNA
Circulogene	Theranostics	ctDNA

CTC: circulating tumor cell; ctDNA: circulating tumor DNA.

REGULATORY STATUS

Table 2 summarizes the targeted treatments approved by the U.S. Food and Drug Administration (FDA) for patients with CRC, along with the approved companion diagnostic tests. The information in Table 2 was current as of June 18, 2021; FDA maintains a list of cleared or approved companion diagnostic devices that is updated regularly.[2,]

Table 2. Targeted Treatments for Metastatic Colorectal Cancer and FDA Approved Companion Diagnostic Tests

Treatment	Indications in Metastatic Colorectal Cancer	Companion Diagnostics
Cetuximab (Erbitux)	*KRAS* wild-type, EGFR-expressing, metastatic colorectal cancer as determined by an FDA-approved test • in combination with FOLFIRI for first-line treatment,	cobas KRAS Mutation Test Dako EGFR pharmDx Kit

	• in combination with irinotecan in patients who are refractory to irinotecan-based chemotherapy, • as a single-agent in patients who have failed oxaliplatin- and irinotecan-based chemotherapy or who are intolerant to irinotecan. Limitations of Use: Erbitux is not indicated for treatment of RAS mutant colorectal cancer or when the results of the RAS mutation tests are unknown	FoundationOne CDx therascreen KRAS RGQ PCR Kit
Panitumumab (Vectibix)	Treatment of wild-type *RAS* (defined as wild-type in both *KRAS* and *NRAS* as determined by an FDA-approved test for this use) metastatic CRC: • In combination with FOLFOX for first-line treatment. • As monotherapy following disease progression after prior treatment with fluoropyrimidine, oxaliplatin, and irinotecan-containing chemotherapy. Limitation of Use: Vectibix is not indicated for the treatment of patients with RAS-mutant mCRC or for whom RAS mutation status is unknown.	cobas KRAS Mutation Test Dako EGFR pharmDx Kit FoundationOne CDx Praxis Extended RAS Panel therascreen KRAS RGQ PCR Kit
Pembrolizumab (Keytruda®)	Unresectable or metastatic, MSI-H or dMMR • solid tumors that have progressed following prior treatment and who have no satisfactory alternative treatment options, or • CRC that has progressed following treatment with a fluoropyrimidine, oxaliplatin, and irinotecan First-line treatment of patients with unresectable or metastatic MSI-H or dMMR CRC	FoundationOne CDx

Source: FDA (2021)[2,]
CRC: colorectal cancer; dMMR: mismatch repair deficient; EGFR: epidermal growth factor receptor; FOLFIRI: leucovorin, fluorouracil and irinotecan; FOLFOX: leucovorin, fluorouracil, and oxaliplatin; HER2: human epidermal growth factor receptor 2; mCRC: metastatic CRC; MSI-H: microsatellite instability-high

Laboratory-Developed Tests

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). Laboratories that offer laboratory-developed tests must be licensed under CLIA for high-complexity testing. To date, the FDA has chosen not to require any regulatory review of this test.

POLICY

A. *KRAS* variant analysis may be considered **medically necessary** for patients with metastatic colorectal cancer to predict nonresponse prior to planned therapy with anti-epidermal growth factor receptor (EGFR) monoclonal antibodies cetuximab or panitumumab.

B. *NRAS* variant analysis may be considered **medically necessary** for patients with metastatic colorectal cancer to predict nonresponse prior to planned therapy with anti-EGFR monoclonal antibodies cetuximab or panitumumab.

C. *BRAF* variant analysis may be considered **medically necessary** for patients with metastatic colorectal cancer who are found to be wild-type on *KRAS* and *NRAS* variant analysis to guide management decisions.

D. Mismatch repair/microsatellite instability (MMR/MSI) testing may be considered **medically necessary** to predict treatment response to pembrolizumab (Keytruda):

1. for first-line treatment of patients with unresectable or metastatic colorectal cancer; **OR**
2. in patients with colorectal cancer that has progressed following treatment with a fluoropyrimidine, oxaliplatin, and irinotecan; **OR**
3. in patients with colorectal cancer tumors that have progressed following prior treatment and who have no satisfactory alternative treatment options.

E. HER2 testing is considered **investigational** to predict treatment response to immunotherapy in patients with metastatic colorectal cancer.

F. Tumor mutational burden testing to predict response to immunotherapy in patients with metastatic colorectal cancer is considered **investigational.**

G. *KRAS, NRAS,* and *BRAF* variant analysis, as well as Mismatch repair/microsatellite instability (MMR/MSI) testing, using circulating tumor DNA or circulating tumor cell testing (liquid biopsy) to guide treatment for patients with metastatic colorectal cancer is considered **experimental / investigational**.

Policy Guidelines

1. There is support from the evidence and clinical input to use *BRAF* V600 variant testing for prognostic stratification.

2. It is uncertain whether the presence of a *BRAF* V600 variant in patients with metastatic colorectal cancer who are wild-type on *KRAS* and *NRAS* variant analysis is predictive of response to anti-epidermal growth factor receptor therapy. Furthermore, there is mixed opinion in clinical guidelines and clinical input on the use of *BRAF* variant analysis to predict response to treatment.

RATIONALE

This evidence review has been updated regularly with searches of the PubMed database. The most recent literature update was performed through June 18, 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.

KRAS Variant Testing to Guide Treatment for Metastatic Colorectal Cancer

Clinical Context and Test Purpose

The purpose of *KRAS* variant testing in individuals with metastatic colorectal cancer (CRC) is to determine *KRAS* variant status to guide treatment decisions with epidermal growth factor receptor (EGFR)-targeted therapy with the monoclonal antibodies cetuximab and panitumumab.
The question addressed in this evidence review is: In individuals with metastatic CRC, does the use of *KRAS* variant testing improve health outcomes?
The following PICO was used to select literature to inform this review.

Populations

The relevant population of interest is individuals with metastatic CRC.

Interventions

The test being considered is *KRAS* variant testing.

Comparators

The following test strategy is currently being used: no *KRAS* variant testing to guide treatment.

Outcomes

The beneficial outcomes of interest include progression-free survival (PFS), overall survival (OS), change in disease status, medication use, resource utilization, and treatment-related morbidity. The time frame for outcomes measures varies from several months to several years.

Study Selection Criteria

For the evaluation of clinical validity, 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;
- 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

This evidence review has been informed, in part, by a TEC Assessment (2008).[3,] Additional evidence derives from systematic reviews, randomized controlled trials (RCTs), and single-arm studies, organized and outlined below.

Randomized Controlled Trials

Randomized controlled trials have performed nonconcurrent subgroup analyses of the efficacy of EGFR inhibitors in patients with wild-type versus mutated *KRAS* in metastatic CRC. Data from these trials have consistently shown a lack of clinical response to cetuximab and panitumumab in patients with mutated *KRAS*, with tumor response and prolongation of PFS observed only in wild-type *KRAS* patients.

Amado et al (2008) performed a subgroup analysis of *KRAS* tumor variants in a patient population that had previously been randomized to panitumumab or to best supportive care as third-line therapy for chemotherapy-refractory metastatic CRC (Table 3).[4,] The original study reported by Van Cutsem et al (2007), designed as a multicenter RCT, was not blinded because of expected skin toxicity related to panitumumab administration.[5,] Patients were randomized 1:1 to panitumumab or to best supportive care. Random assignment was stratified by Eastern Cooperative Oncology Group (ECOG) Performance Status (0 or 1 vs 2) and geographic region. Crossover from best supportive care to the panitumumab arm was allowed in patients who experienced disease progression. Of the 232 patients originally assigned to best supportive care alone, 176 crossed over to the panitumumab arm, at a median time to a crossover of 7 weeks (range, 6.6-7.3 weeks).

Of the 463 patients in the original trial, 427 (92%) were included in the *KRAS* subgroup variant analysis. A central laboratory performed the *KRAS* variant analysis in a blinded fashion, using formalin-fixed, paraffin-embedded tumor sections and a validated *KRAS* variant kit (DxS) that identifies 7 somatic variants located in codons 12 and 13 using real-time polymerase chain reaction. *KRAS* variant status could not be determined in 36 patients because tumor samples were not available or DNA was of insufficient or poor quality for analysis. Forty-three percent of the *KRAS*-evaluable patients had *KRAS*-mutated tumors, with a distribution similar to *KRAS* variant types between treatment arms.

Patient demographics and baseline characteristics were balanced between the wild-type and mutated groups for the panitumumab and best supportive care groups including patient age, sex, and ECOG Performance Status. The interaction between variant status and PFS was examined, controlling for randomization factors. Progression free survival and tumor response rate were assessed radiographically every 4 to 8 weeks until disease progression using Response Evaluation Criteria in Solid Tumors by blinded, central review. In the *KRAS*-assessable population, 20% of patients had a treatment-related grade 3 or 4 adverse event. As shown in Table 3, the relative effect of panitumumab on PFS was significantly greater among patients with wild-type *KRAS* than patients with mutated *KRAS* in whom no benefit from panitumumab was observed. No responders to panitumumab were identified in the mutated group, indicating a 100% positive predictive value for nonresponse in that group.

Table 3. *KRAS* Status and Efficacy of Panitumumab as Monotherapy in the Treatment of Chemotherapy-Refractory Metastatic Colorectal Cancer (n=427)

Outcomes	***KRAS* WT (n=243 [57%])**		***KRAS* MT (n=184 [43%])**	
	Panitumumab (n=124)	Best supportive care (n=119)	Panitumumab (n=84)	Best supportive care (n=100)
Median progression-free survival, weeks	12.3	7.3	7.4	7.3
Hazard ratio (95% CI)	0.45 (0.34 to 0.59)		0.99 (0.73 to 1.36)	
Response rate, %	17		0	

Adapted from Amado et al (2008).[4,]
CI: confidence interval; MT: mutated; WT: wild-type.

Given the crossover trial design and the fact that most of the best supportive care patients crossed over to the panitumumab arm early in the trial, conclusions on the effect of *KRAS* variant status on PFS and tumor response rate endpoints are limited. However, of the 168 best supportive care patients who crossed over to panitumumab after disease progression (119 with wild-type *KRAS*, 77 with mutated *KRAS*), PFS was significantly longer among patients with wild-

type *KRAS* (median PFS: 16.4 weeks for wild-type versus 7.9 weeks for mutated; hazard ratio [HR], 0.32; 95% confidence interval [CI], 0.22 to 0.45).

After completion of the Cetuximab Combined with Irinotecan in First-Line Therapy for Metastatic CRC (CRYSTAL) trial (detailed below), in which 1198 patients with metastatic CRC were randomized to cetuximab in combination with folinic acid (leucovorin), 5-florouracil, and irinotecan (FOLFIRI) or to FOLFIRI alone for first-line treatment, a subgroup analysis of response rate and PFS by *KRAS* variant status was performed by Van Cutsem et al (2009).[6,] The original trial design consisted of a central stratified permuted block randomization procedure with geographic regions and ECOG Performance Status as randomization strata. Two interim assessments of safety data were conducted by an independent data safety monitoring board. Of the original 1198 patients, 540 had *KRAS*-evaluable, archival material. *KRAS* testing was performed using genomic DNA isolated from archived formalin-fixed, paraffin-embedded tissue, using quantitative polymerase chain reaction to detect the *KRAS* variant status of codons 12 and 13. It was not stated whether the *KRAS* variant analysis was performed blinded. *KRAS* variants were present in 192 (35.6%) patients. No differences were found in patient demographics or baseline characteristics between the mutated and wild-type populations, including age, sex, ECOG Performance Status, involved disease sites, and liver-limited disease. Tumor response rate and PFS were assessed by a blinded, independent review committee using computed tomography scans every 8 weeks. A multivariate analysis performed for PFS by patient characteristics showed a trend for PFS favoring the cetuximab plus FOLFIRI combination. The patients with wild-type *KRAS* who received cetuximab plus FOLFIRI showed a statistically significant improvement in median PFS and tumor response rate, whereas the mutated *KRAS* population did not, as summarized in Table 4.

Table 4. *KRAS* Status and Efficacy in the First-Line Therapy of Metastatic Colorectal Cancer Treated With FOLFIRI With or Without Cetuximab (CRYSTAL Trial) (n=540)

Outcomes	**ITT[a]**		***KRAS* WT (n=348 [64%][b])**		***KRAS* MT (n=192 [36%][b])**	
	C+F	F	C+F	F	C+F	F
n	599	599	172	176	105	87
RR (95% CI), %	46.9 (42.9 to 51.0)	38.7 (34.8 to 42.8)	59.3 (51.6 to 66.7)	43.2 (35.8 to 50.9)	36.2 (27.0 to 46.2)	40.2 (29.9 to 51.3)
Median PFS, months[c]	8.9	8.0	9.9	8.7	7.6	8.1
Hazard ratio (p-value)			0.68 (p=.017)		1.07 (p=.47)	

Adapted from Van Cutsem et al (2009).[6,]

C: cetuximab; CI: confidence interval; F: FOLFIRI (folinic acid, 5-florouracil, and irinotecan); ITT: intention-to-treat; MT: mutated; PFS: progression-free survival; RR: response rate; WT: wild-type.

[a] ITT in the original CRYSTAL trial assessing C+F vs F alone as first-line therapy for metastatic colorectal cancers.

[b] 540 patients had available archival pathology material for the *KRAS* variant subset analysis.

[c] Confidence intervals for median PFS were not provided in the presentation slides.

In a third trial, the phase 2 Oxaliplatin and Cetuximab in First-Line Treatment of metastatic CRC (OPUS) trial, the intention-to-treat (ITT) population consisted of 337 patients randomized to cetuximab and folinic acid (leucovorin), 5-florouracil, and oxaliplatin (FOLFOX) or to FOLFOX alone in the first-line treatment of metastatic CRC.[7,] A 10% higher response rate (assessed by independent reviewers) was observed in the population treated with cetuximab, but no difference in PFS was seen between groups. Researchers then reevaluated the efficacy in the 2 treatment

arms based on the *KRAS* variant status of patients' tumors. Of the original ITT population, 233 subjects had evaluable material for *KRAS* testing, and 99 (42%) were *KRAS* variants. The demographics were similar between the wild-type and mutated groups, including patient age, sex, ECOG Performance Status, involved disease sites, and liver-limited disease. The trial showed that the addition of cetuximab to FOLFOX resulted in a significant improvement in response rate and PFS only in the wild-type *KRAS* group. Table 5 summarizes study findings.

Table 5. *KRAS* Status and Efficacy in the First-Line Therapy of Metastatic Colorectal Cancer Treated With FOLFOX With or Without Cetuximab (OPUS Study) (n=233)

Outcomes	***KRAS* WT (n=134 [58%])**		***KRAS* MT (n=99 [42%])**	
	C+Fx	**Fx**	**C+Fx**	**Fx**
n (*KRAS*-evaluable)	61	73	52	47
RR (95% CI), %	60.7 (47.3 to 72.9)	37.0 (26.0 to 49.1)	32.7 (20.3 to 47.1)	48.9 (34.1 to 63.9)
p-value	.011		.106	
Odds ratio (95% CI)	2.54 (1.24 to 5.23)		0.51 (0.22 to 1.15)	
Median PFS, mo[a]	7.7	7.2	5.5	8.6
p	.016		.019	
Hazard ratio	0.57		1.83	

Adapted from Bokemeyeret al (2009).[7,]
C: cetuximab; CI: confidence interval; Fx: FOLFOX (folinic acid, 5-florouracil, and oxaliplatin); MT: mutated; PFS: progression-free survival; RR: response rate; WT: wild-type.
[a] Confidence intervals for median PFS were not provided in presentation slides.

In the addition of cetuximab to capecitabine, oxaliplatin, and bevacizumab as first-line treatment in patients with mCRC (CAIRO2) study, Tol et al (2009) analyzed tumor samples from 528 of 755 previously untreated patients with metastatic CRC who were randomized to capecitabine, oxaliplatin, and bevacizumab (CB regimen, n=378), or to the same CB regimen plus cetuximab (n=377).[8,] *KRAS* variant was found in 40% of tumors (108 from patients in the CB group, 98 from the CB plus cetuximab group). Patients with *KRAS* variants treated with cetuximab had a significantly shorter PFS (8.1 months) than the wild-type *KRAS* patients who received cetuximab (10.5 months; p=.04). In addition, patients who had mutated *KRAS* tumors who received cetuximab had a significantly shorter PFS and OS than patients with mutated *KRAS* tumors who did not receive cetuximab (PFS: 8.1 months vs 12.5 months, respectively, p=.003; OS: 17.2 months vs 24.9 months, respectively, p=.03). For patients with wild-type tumors, no significant PFS differences were reported between groups. Overall, patients treated with cetuximab who had tumors with a mutated *KRAS* gene had significantly decreased PFS compared with cetuximab-treated patients with wild-type *KRAS* tumors or patients with mutated *KRAS* tumors in the CB group.

Karapetis et al (2008) analyzed tumor samples from 394 (69%) of 572 patients with CRC who were randomized to cetuximab plus best supportive care (n=287) or to best supportive care alone (n=285) for *KRAS* variants and assessed whether variant status was associated with survival.[9,] The patients with advanced CRC had failed chemotherapy and had no other standard anticancer therapy available. Of the tumors evaluated (198 from the cetuximab group, 196 from the best supportive care group), 41% and 42% had a *KRAS* variant, respectively. These groups reported a median OS of 9.5 months and 4.8 months, respectively (HR for death, 0.55; 95% CI, 0.41 to 0.74; p<.001) and a median PFS of 3.7 months and 1.9 months, respectively (HR for

progression to death, 0.40; 95% CI, 0.30 to 0.54; p<.001). For patients with mutated *KRAS* tumors, no significant differences were reported between those treated with cetuximab and best supportive care alone with respect to OS (HR=0.98, p=.89) or PFS (HR=0.99, p=.96).

Douillard et al (2010) reported on the results of a multicenter, phase 3 trial in which patients with no prior chemotherapy for metastatic CRC, ECOG Performance Status of 0 to 2, and available tissue for biomarker testing were randomized 1:1 to panitumumab plus FOLFOX4 (folinic acid, fluorouracil, and oxaliplatin; 4 refers to dosing regimen) or to FOLFOX4.[10,] The primary endpoint was PFS; OS was a secondary endpoint. Results were prospectively analyzed on an ITT basis by tumor *KRAS* status. *KRAS* results were available for 93% of the 1183 patients randomized. In the wild-type *KRAS* group, panitumumab plus FOLFOX4 significantly improved PFS compared with FOLFOX4 alone (median PFS, 9.6 months vs 8.0 months, respectively; HR=0.80; 95% CI, 0.66 to 0.97; p=.02). A nonsignificant increase in OS was also observed for panitumumab plus FOLFOX4 versus FOLFOX4 (median OS, 23.9 months vs 19.7 months, respectively; HR=0.83; 95% CI, 0.67 to 1.02; p=.072). In the mutant *KRAS* group, PFS was significantly reduced in the panitumumab plus FOLFOX4 arm compared with the FOLFOX4 arm (HR=1.29; 95% CI, 1.04 to 1.62; p=.02), and median OS was 15.5 months versus 19.3 months, respectively (HR=1.24; 95% CI, 0.98 to 1.57; p=.068). Adverse event rates were generally comparable across arms with the exception of toxicities known to be associated with anti-EGFR therapy. The trial demonstrated that panitumumab plus FOLFOX4 was well-tolerated and significantly improved PFS in patients with wild-type *KRAS* tumors.

The CRYSTAL trial (2009), mentioned above, demonstrated that the addition of cetuximab to FOLFIRI significantly reduced the risk of disease progression and increased the chance of response in patients with wild-type *KRAS* metastatic CRC compared with chemotherapy alone.[6,] In an updated analysis of CRYSTAL, Van Cutsem et al (2011) reported on longer follow-up and more patients evaluable for tumor *KRAS* status and considered the clinical significance of the *BRAF* variant tumor status in the expanded population of patients with wild-type *KRAS* tumors.[11,] Subsequent to the initial published analysis, which reported an OS cutoff of December 2007 and an associated overall median duration of follow-up of 29.7 months, additional tumor analysis allowed for the typing of another 523 tumors for *KRAS* variant status, representing an increase in the ascertainment rate from 45% of ITT population patients in the original analysis to 89% (540 to 1063) in the current analysis, with variants detected in 37% of tumors. The updated OS analysis was carried out with a new cutoff date of May 2009, giving an overall median duration of follow-up of 46 months. The addition of cetuximab to FOLFIRI in patients with wild-type *KRAS* disease resulted in significant improvements in OS (median, 23.5 months vs 20.0 months; HR=0.796; p=.009), PFS (median, 9.9 months vs 8.4 months; HR=0.696; p=.001), and response rate (57.3% vs 39.7%; odds ratio [OR], 2.069; p<.001) compared with FOLFIRI alone. Significant interactions between *KRAS* status and treatment effect were noted for all key efficacy endpoints. *KRAS* variant status was confirmed as a powerful predictive biomarker for the efficacy of cetuximab plus FOLFIRI. *BRAF* V600E variants were detected in 60 (6%) of 999 tumor samples evaluable for both *BRAF* and *KRAS*. In all but a single case, *BRAF* variants were identified in tumors wild-type for *KRAS*. The impact of *BRAF* tumor variant status in relation to the efficacy of cetuximab plus FOLFIRI was examined in the population of patients with wild-type *KRAS* disease (n=625). No evidence was reported for an independent treatment interaction by tumor *BRAF* variant status. The trialists concluded that *BRAF* variant status was not predictive of treatment effects of cetuximab plus FOLFIRI but that *BRAF* tumor variant was a strong indicator of poor prognosis for all efficacy endpoints compared with those whose tumors were wild-type.

Peeters et al (2010) reported on the results of a phase 3 study in which 1186 patients with metastatic CRC were randomized to panitumumab plus FOLFIRI or to FORFIRI alone as a second-line treatment.[12,] The trial endpoints were PFS and OS, which were independently tested and prospectively analyzed by *KRAS* status. *KRAS* status was available for 91% of patients: 597 (55%) had wild-type *KRAS* tumors and 486 (45%) had mutated *KRAS* tumors. In the wild-type *KRAS* subpopulation, when panitumumab was added to chemotherapy, a significant improvement in PFS was observed (HR=0.73; 95% CI, 0.59 to 0.90; p=.004); median PFS was 5.9 months for panitumumab plus FOLFIRI and 3.9 months for FOLFIRI. A nonsignificant trend toward increased OS was observed; median OS for panitumumab plus FOLFIRI was 14.5 months while median OS for FOLFIRI alone was 12.5 months (HR=0.85, 95% CI, 0.70 to 1.04; p=.12). Response rates improved with the addition of panitumumab to the FOLFIRI regimen. In patients with mutated *KRAS*, no difference was reported in efficacy. Adverse events were comparable across arms. The trialists concluded that panitumumab plus FOLFIRI significantly improved PFS and was well-tolerated as second-line treatment in patients with wild-type *KRAS* metastatic CRC.
Maughan et al (2011) reported on the results of a phase 3, multicenter trial, which randomized patients with advanced CRC who had not received previous chemotherapy to oxaliplatin plus fluoropyrimidine chemotherapy (arm A) or to the same combination plus cetuximab (arm B).[13,] The comparison between arms A and B (for which the primary outcome was OS) was in patients with wild-type *KRAS* tumors. Baseline characteristics were well-balanced between groups. The analysis was by ITT and treatment allocation was not masked. A total of 1630 patients were randomized to treatment groups (815 to standard therapy, 815 to the addition of cetuximab). Tumor samples from 1316 (81%) patients were used for somatic variant analyses; 43% had *KRAS* variants. In patients with wild-type *KRAS* tumors, OS did not differ between treatment groups (median survival, 17.9 months in the control group vs 17.0 months in the cetuximab group; HR=1.04; 95% CI, 0.87 to 1.23; p=.67). *BRAF* variants were detected in 8% of patients; *BRAF* did not show any evidence of a benefit from the addition of cetuximab. Contrary to other trials that have studied the benefit of adding cetuximab to the regimen of wild-type *KRAS* patients, this trial did not show a benefit of adding cetuximab to oxaliplatin-based chemotherapy.

Systematic Reviews

Qiu et al (2010) conducted a meta-analysis of 22 studies on the predictive and prognostic value of *KRAS* variants in metastatic CRC patients treated with cetuximab.[14,] The overall *KRAS* variant rate was 38% (829/2188 patients). Meta-analytic results were consistent with previous studies on the use of cetuximab and *KRAS* variant status, in that patients with tumors harboring mutant-type *KRAS* were more likely to have a worse response, PFS, and OS when treated with cetuximab than those with wild-type *KRAS*.
Dahabreh et al (2011) conducted a systematic review of RCTs that assessed the use of *KRAS* variant testing as a predictive biomarker for treatment of advanced CRC with cetuximab and panitumumab.[15,] Reviewers concluded that, compared with patients who had wild-type *KRAS*, *KRAS* variants were consistently associated with reduced OS and PFS and increased treatment failure rates among patients with advanced CRC who are treated with anti-EGFR antibodies.
In a pooled analysis of wild-type *KRAS* tumors from the CRYSTAL and OPUS trials, Bokemeyer et al (2012) assessed extended survival data and enhancement in the ascertainment rate of *KRAS* and *BRAF* tumor variant status.[16,] Pooled individual patient data from each trial were analyzed for OS, PFS, and best objective response rate (ORR) in patients evaluable for *KRAS* and *BRAF* variant status. In 845 patients with wild-type *KRAS* tumors, adding cetuximab to chemotherapy led to significant improvements in OS (HR=0.81; p=.006), PFS (HR=0.66;

p<.001), and ORR (OR=2.16; p<.001). *BRAF* variants were detected in 70 (8.8%) of 800 evaluable tumors. No significant differences were found in outcomes between treatment groups. However, the prognosis was worse in each treatment arm for patients with *BRAF* tumors, and OPUS trials confirmed the consistency of the benefit obtained from all efficacy endpoints from adding cetuximab to first-line chemotherapy in patients with wild-type *KRAS* metastatic CRC. It further suggested that *BRAF* variants do not appear to be predictive biomarkers in this setting but are markers of poor prognosis.

Single-Arm Studies

In addition to the randomized trials discussed, a number of single-arm studies have retrospectively evaluated *KRAS* variant status and treatment response in patients with metastatic CRC.[17,18,19,20,21,] Overall they have shown similar nonresponse rates to anti-EGFR monoclonal antibodies (cetuximab, panitumumab) in patients with mutated *KRAS* tumors. Two of these single-arm studies have also reported differences in PFS and OS.[18,21,]

Clinically Useful

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

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 RCTs.

No RCTs were identified on the clinical utility of *KRAS* variant testing to predict nonresponse to anti-EGFR monoclonal antibody therapy.

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, based on clinical validity, supports the use of the anti-EGFR monoclonal antibodies cetuximab and panitumumab for the treatment of patients with wild-type *KRAS* metastatic CRC. Cetuximab and panitumumab are not indicated for the treatment of patients when *KRAS* variants are present or when *KRAS* variant status is unknown.

Section Summary: *KRAS* Variant Testing to Guide Treatment for Metastatic Colorectal Cancer

Evidence for the clinical validity of *KRAS* variants in predicting nonresponse to anti-EGFR monoclonal antibody therapy consists of multiple systematic reviews, including a TEC Assessment, and RCTs. The evidence has demonstrated that the presence of a *KRAS* variant predicts nonresponse to treatment, while *KRAS* wild-type status predicts response to anti-EGFR monoclonal antibody therapy. Direct evidence for the clinical validity of *KRAS* variant testing includes RCTs. Randomized controlled trials supporting U.S. Food and Drug Administration approvals for cetuximab and panitumumab have demonstrated that the presence of *KRAS* variants is predictive of nonresponse to anti-EGFR monoclonal antibody therapy. Documentation of *KRAS* wild-type status is required before patients are eligible for treatment with cetuximab or panitumumab.

***NRAS* Variant Testing to Guide Treatment for Metastatic Colorectal Cancer**

Clinical Context and Test Purpose

The purpose of *NRAS* variant testing in individuals with metastatic CRC is to determine *NRAS* variant status to guide treatment decisions with EGFR-targeted therapy with the monoclonal antibodies cetuximab and panitumumab.

The question addressed in this evidence review is: In individuals with metastatic CRC, does the use of *NRAS* variant testing improve health outcomes?
The following PICO was used to select literature to inform this review.

Populations

The relevant population of interest is individuals with metastatic CRC.

Interventions

The test being considered is *NRAS* variant testing.

Comparators

The following test strategy is currently being used: no *NRAS* variant testing to guide treatment.

Outcomes

The beneficial outcomes of interest include PFS, OS, change in disease status, medication use, resource utilization, and treatment-related morbidity.
The time frame for outcomes measures varies from several months to several years.

Study Selection Criteria

For the evaluation of clinical validity, 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;
- Patient/sample clinical characteristics were described;
- Patient/sample selection criteria were described.

Clinically Valid

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

Review of Evidence

Systematic Reviews

A systematic review by Therkildsen et al (2014) evaluated the predictive value of *NRAS* variants on clinical outcomes of anti-EGFR therapy in CRC.[22,] The meta-analysis included data from 3 studies described below.[23,24,25,] Reviewers suggested that the pooled analyses showed a trend toward a poor overall response based on 17 events, but significant effects on PFS (HR=2.30; 95% CI, 1.30 to 4.07) and OS (HR=1.85; 95% CI, 1.23 to 2.78) among patients with wild-type *KRAS*. These results are limited by the small pool of variants, permitting no conclusions whether *NRAS* variants have an effect on anti-EGFR therapy.

Prospective-Retrospective Analyses of Randomized Controlled Trials

Randomized controlled trials have analyzed nonconcurrent subgroups for the efficacy of EGFR inhibitors in patients with wild-type and mutated *RAS* genes in metastatic CRC.
Peeters et al (2015) reported on the influence of *RAS* variant status in a prospective-retrospective analysis of a randomized, multicenter phase 3 trial comparing panitumumab plus FOLFIRI with FOLFIRI alone as second-line therapy in patients with metastatic CRC.[26,] If a tumor was classified as wild-type *KRAS* exon 2, extended *RAS* variant testing beyond *KRAS* exon 2 was performed (*KRAS* exons 3 and 4; *NRAS* exons 2, 3, and 4; *BRAF* exon 15). Primary endpoints were PFS and OS. *RAS* variants were obtained in 85% of the specimens from the original trial; 18% of wild-type *KRAS* exon 2 tumors harbored other *RAS* variants. Table 6 summarizes the PFS and OS HRs for panitumumab plus FOLFIRI versus FOLIRI alone. The HRs more strongly favored panitumumab in the wild-type *RAS* population.

Table 6. Hazard Ratios of Panitumumab Plus FOLFIRI versus FOLFIRI Alone Based on *RAS* Status

RAS Status	PFS HR (95% CI)	p-value	OS HR (95% CI)	p-value

Wild-type *RAS*	0.70 (0.54 to 0.91)	.007	0.81 (0.63 to 1.03)	.08
Wild-type *KRAS* exon 2	0.73 (0.59 to 0.90)	.004	0.85 (0.70 to 1.04)	.12

CI: confidence interval; FOLFIRI: (folinic acid, 5-florouracil, and irinotecan); HR: hazard ratio; OS: overall survival; PFS: progression-free survival.

For *RAS* wild-type patients, the ORR was 41% when patients were treated with panitumumab plus FOLFIRI versus 10% when treated with FOLFIRI alone. Therefore, *RAS* wild-type status predicted a likely response to panitumumab and overall benefit from treatment. In contrast, the presence of *RAS* variants predicted nonresponse to panitumumab and unlikely benefit from treatment.

Van Cutsem et al (2015) reported on results of a prospective-retrospective extended *RAS* variant analysis of tumor samples from the randomized phase 3 CRYSTAL trial, which compared FOLFIRI with FOLFIRI plus cetuximab in wild-type *KRAS* exon 2 patients.[27,] Variant status was available in 430 (64.6%) of 666 patients from the trial. A pooled analysis of *RAS* variants, other than *KRAS* exon 2, found a lack of benefit from the addition of cetuximab to FOLFIRI for median PFS (7.4 months vs 7.5 months; p=.47) and median OS (16.4 months vs 17.7 months; p=.64). Patients with tumors without *RAS* variants experienced a significant benefit in median PFS (9.9 months vs 8.4 months; p<.05) and median OS (23.5 months vs 20 months; p<.05) with the addition of cetuximab to chemotherapy.

Douillard et al (2013) performed a prospective-retrospective analysis of *RAS* variants (*KRAS*, *NRAS*) in tumor samples from patients enrolled in the Panitumumab Randomized Trial in Combination with Chemotherapy for Metastatic Colorectal Cancer to Determine Efficacy RCT.[23,] A total of 108 (17%) of 641 tumor specimens that did not harbor exon 2 *KRAS* variants had variants in other *RAS* exons, including *NRAS* (exons 2 or 4) and *KRAS* (exons 3 and 4). For patients with a wild-type *KRAS* exon 2 variant (n=656), OS was significantly better with panitumumab plus FOLFOX4 (n=325; median, 23.8 months) than with FOLFOX4 alone (n=331; median, 19.4 months; p=.03). For patients with no *KRAS* exon 2 variant but with 1 type of *RAS* variant, median OS with panitumumab plus FOLFOX4 was shorter (n=51; median, 17.1 months) than with FOLFOX4 alone (n=57; median, 17.8 months; p=.01). These data would suggest variants in a *RAS* gene exon other than *KRAS* exon 2 negatively affect anti-EGFR therapy. However, the investigators did not discriminate between specific types of *RAS* variants, so it is not possible to relate *NRAS* to these results. Furthermore, the numbers of patients involved were very small, further limiting conclusions.

Tumor specimens (288 of 320) from an RCT by Van Cutsem et al (2007)[5,] were analyzed by Peeters et al (2013) using next-generation sequencing to investigate whether EGFR pathway variants would predict response to monotherapy with panitumumab compared with best supportive care.[24,] This 2013 analysis showed that *NRAS* had mutated in 14 (5%) of 282 samples with available data. Among patients with wild-type *KRAS* (codons 12, 13, and 61) and wild-type *NRAS* (n=138), treatment with panitumumab was associated with improved PFS (HR=0.39; 95% CI, 0.27 to 0.56; p<.001) compared with best supportive care. Among those with wild-type *KRAS* but mutated *NRAS* (n=11), treatment with panitumumab was no longer associated with longer PFS (HR=1.94; 95% CI, 0.44 to 8.44; p=.379). A treatment interaction analysis was suggestive but not significantly indicative of an interaction between the presence of mutated *NRAS* and poorer outcome (p=.076). The authors suggested their data were consistent with the hypothesis that *NRAS* variants may limit the efficacy of anti-EGFR therapy. However, because the prevalence of *NRAS* variants was low, the degree of predictive or prognostic value is more uncertain.

Retrospective Cohort Studies

A retrospective consortium analysis by De Roock et al (2010) reported on results of centrally performed high-throughput mass spectrometric variant profiling of CRC specimens gathered from 11 centers in 7 European countries.[25,] Patients had been treated with panitumumab alone, cetuximab alone, or cetuximab plus chemotherapy. Among 747 of 773 samples with data, *KRAS* had mutated in 299 (40%), including codons 12, 13, 61, and 146. By contrast, *NRAS* variants were identified in 17 (2.6%) of 644 samples with data, primarily in codon 61. *KRAS* and *NRAS* variants were mutually exclusive. Among wild-type *KRAS* samples from patients treated with cetuximab plus chemotherapy, the *NRAS* variant was associated with an ORR of 7.7% (1/13) compared with 38% for the wild-type *NRAS* (p=.013). However, there were no significant differences between *NRAS* mutant and wild-type genes in median PFS (14 weeks vs 26 weeks, p=.055) or OS (38 weeks vs 50 weeks, p=.051). Similar to results previously reported, the results of this analysis showed a very low prevalence of *NRAS* variants and were inconclusive as to whether *NRAS* variants are predictive of nonresponse to anti-EGFR therapy or are prognostic indicators of poor outcomes of CRC.

The rarity of *NRAS* variants reported in the studies discussed was also shown in a study by Irahara et al (2010) that used polymerase chain reaction and pyrosequencing (Qiagen) to assess tumor samples from individuals who developed CRC and were identified within the databases of 2 prospective cohort studies: the Nurses' Health Study and the Health Professionals Follow-Up Study.[28,] Among 225 CRC specimens, *NRAS* variants were identified in 5 (2.2%). Because of the low frequency of *NRAS* variants, they were not associated with any clinical or pathologic features or with patient survival.

Clinically Useful

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

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 RCTs.

No RCTs were identified on the clinical utility of *NRAS* variant testing to predict nonresponse to anti-EGFR monoclonal antibody therapy.

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.

Documentation of *KRAS* wild-type status is required prior to treatment with cetuximab or panitumumab.

A chain of evidence, based on clinical validity, supports the use of the anti-EGFR monoclonal antibodies cetuximab and panitumumab for the treatment of patients with wild-type *NRAS* metastatic CRC. Documentation of *NRAS* variant status is not required but has been recommended to identify patients who are predicted to be nonresponders to anti-EGFR monoclonal antibody therapy.

Section Summary: *NRAS* Variant Testing to Guide Treatment for Metastatic Colorectal Cancer

Evidence for the clinical validity of *NRAS* variants in predicting nonresponse to anti-EGFR monoclonal antibody therapy includes prospective-retrospective analyses of RCTs. Subgroup analyses of *KRAS* wild-type patients who did not respond to anti-EGFR monoclonal antibody therapy have suggested that *NRAS* variants are predictive of nonresponse. However, because of the low prevalence of *NRAS* variants, the predictive value of *NRAS* variants is uncertain. Direct

evidence for the clinical utility of *NRAS* variant testing includes prospective-retrospective analyses of RCTs and retrospective cohort studies. *NRAS* variant testing has potential clinical utility in predicting nonresponse to anti-EGFR monoclonal antibody therapy in patients with documented *KRAS* wild-type status. However, the direct evidence is limited for *NRAS* variant testing due to low prevalence *NRAS* variants in CRC.

BRAF Variant Testing to Guide Treatment for Metastatic Colorectal Cancer

Clinical Context and Test Purpose

The purpose of *BRAF* variant testing in individuals with metastatic CRC is to determine *BRAF* variant status to guide treatment.

The question addressed in this evidence review is: In individuals with metastatic CRC, does the use of *BRAF* variant testing improve health outcomes?

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

Populations

The relevant population of interest is individuals with metastatic CRC who are found to be wild-type on *KRAS* and *NRAS* variant analysis.

Interventions

The test being considered is *BRAF* variant testing.

Comparators

The following test strategy is currently being used: no *BRAF* variant testing to guide management.

Outcomes

The beneficial outcomes of interest include PFS, OS, change in disease status, medication use, resource utilization, and treatment-related morbidity.

The time frame for outcomes measures varies from several months to several years.

Study Selection Criteria

For the evaluation of clinical validity, 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;
- 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

Systematic Reviews

A meta-analysis by Pietrantonio et al (2015) identified 9, phase 3 trials that compared cetuximab or panitumumab with standard therapy or best supportive care.[29,] The analysis included 463 patients with metastatic CRC and *BRAF* variants. The addition of an EGFR inhibitor did not improve PFS (HR=0.88; 95% CI, 0.67 to 1.14; p=.33) or ORR (RR=1.31; 95% CI, 0.83 to 2.08; p=.25) compared with the control arms.

A meta-analysis by Mao et al (2011) assessed *BRAF* variants and resistance to anti-EGFR monoclonal antibodies in patients with metastatic CRC.[30,] The primary endpoint of eligible studies was ORR, defined as the sum of complete and partial tumor response. Eleven studies reported sample sizes ranging from 31 to 259 patients.[31,32,33,34,35,36,37,38,39,40,] All were conducted retrospectively (1 study was a nonconcurrent analysis of response in a population previously randomized[40,]). Anti-EGFR therapy was given as first-line treatment in 1 study and as second-line or greater in the other 10. In 2 studies, the anti-EGFR monoclonal antibody was given as

monotherapy, and in 9 studies, patients received various chemotherapies. Seven studies were performed in unselected patients (ie, unknown *KRAS* variant status) totaling 546 patients, for whom 520 were assessable for tumor response. In the unselected population, a *BRAF* variant was detected in 8.8% of patients, and the ORR for patients with mutant *BRAF* was 29.2% (14/48) and for wild-type *BRAF* was 33.5% (158/472; p=.048). Four studies were performed in patients with wild-type *KRAS* metastatic CRC. *BRAF* variant status was performed on 376 wild-type *KRAS* tumors. *BRAF* variant was detected in 10.6% (n=40) of primary tumors. Among the 376 analyzed, all patients were assessable for tumor response. The ORR of patients with a mutant *BRAF* gene was 0% (0/40), whereas the ORR of patients with wild-type *BRAF* was 36.3% (122/336). Only 3 studies presented data on PFS and OS and, therefore, a pooled analysis was not performed. Reviewers concluded that, although the meta-analysis provided evidence that *BRAF* variants were associated with lack of response to anti-EGFR monoclonal antibodies in wild-type *KRAS* metastatic CRC, the number of studies and number of patients analyzed were relatively small and that large studies would be needed to confirm the meta-analytic results using homogenous metastatic CRC patients with assessors blinded to the clinical data.
Mao et al (2011) meta-analysis also assessed *BRAF* V600E variant and resistance to anti-EGFR monoclonal antibodies in patients with metastatic CRC.[30,] The same 11 studies were selected. Seven included unselected patients, and 4 studies included only patients with wild-type *KRAS*. The primary endpoint was ORR. In the 7 studies with unselected patients, *BRAF* variant status was performed successfully on 546 metastatic CRC. *BRAF* variants were detected in 8.8% of primary tumors. The ORR of metastatic CRC patients with mutant *BRAF* was 29.2% and 33.5% in patients with wild-type *BRAF*. In the 4 studies that included patients with wild-type *KRAS*, *BRAF* variant status was performed successfully on 376 wild-type *KRAS* metastatic CRC. *BRAF* variants were detected in 10.6% of primary tumors. The ORR of patients with mutant *BRAF* genes was 0.0%, whereas it was 36.3% in patients with wild-type. Reviewers concluded that their results provided evidence that the *BRAF* variant is associated with lack of response in wild-type *KRAS* metastatic CRC treated with anti-EGFR monoclonal antibodies.

Retrospective Studies

Di Nicolantonio et al (2008) retrospectively analyzed 113 patients with metastatic CRC who had received cetuximab or panitumumab.[32,] None of the *BRAF*-mutated tumors (0/11) responded to treatment, whereas 32.4% (22/68) of the wild-type *BRAF* did. Loupakis et al (2009) retrospectively assessed 87 patients receiving irinotecan and cetuximab.[35,] Of the 87 patients in the study, *BRAF* was mutated in 13 patients, of whom none responded to chemotherapy, compared with 32% (24/74) of patients with wild-type *BRAF* who did. In the CAIRO2 study, Tol et al (2009) retrospective analyzed *BRAF* variants in 516 available tumors from patients previously randomized to the CB regimen or to the CB plus cetuximab regimen.[40,] A *BRAF* variant was found in 8.7% (n=45) of the tumors. Patients with a *BRAF* variant had a shorter median PFS and OS compared with wild-type *BRAF* tumors in both treatment arms. The authors concluded that a *BRAF* variant was a negative prognostic marker in patients with metastatic CRC and that this effect, unlike *KRAS* variants, was not restricted to the outcome of cetuximab treatment. In the CRYSTAL trial, Van Cutsem et al (2009) randomized 1198 patients with untreated metastatic CRC to FOLFIRI with or without cetuximab.[6,] Analysis of *BRAF* variants in this patient population and the influence of *BRAF* variant status by Peeters et al (2014) showed that for the wild-type, *KRAS*- and *BRAF*-mutated patients, OS for cetuximab plus FOLFIRI was 14.1 months and 10.3 months with FOLFIRI (p=.744).[41,] Although this difference was not statistically significant, it suggested a trend toward improved OS, PFS, and response, and that wild-type *KRAS*- and *BRAF*-mutant patients might benefit from anti-EGFR therapy.

De Roock et al (2010) reported on the effects of 4 variants, including *BRAF*, on the efficacy of cetuximab and chemotherapy in chemotherapy-refractory metastatic CRC in 773 primary tumor samples.[25,] Tumor samples were from fresh frozen or formalin-fixed, paraffin-embedded tissue, and the variant status was compared with retrospectively collected clinical outcomes including ORR, PFS, and OS. *BRAF* variants were found in 36 (4.7%) of 761 tumors. In patients with wild-type *KRAS*, carriers of *BRAF* variants had a significantly lower response rate (8.3% [2/24] patients) than wild-type *BRAF* (38.0% [124/326] patients; OR=0.15; 95% CI, 0.02 to 0.51; p=.001). Progression free survival for *BRAF*-mutated versus wild-type patients was a median of 8 weeks versus 26 weeks, respectively (HR=3.74; 95% CI, 2.44 to 5.75; p<.001), and median OS was 26 weeks versus 54 weeks, respectively (HR=3.03; 95% CI, 1.98 to 4.63; p<.001).
In an updated analysis of the CRYSTAL trial, Van Cutsem et al (2011) reported on longer follow-up and more patients with evaluable for *KRAS* tumor status and considered the clinical significance of *BRAF* tumor variant status in the expanded population of patients with wild-type *KRAS* tumors.[11,] The impact of *BRAF* tumor variant status on the efficacy of cetuximab plus FOLFIRI was examined in the population with wild-type *KRAS* disease (n=625). No evidence was reported for an independent treatment interaction by *BRAF* tumor variant status. The authors concluded that *BRAF* variant status was not predictive of the treatment effects of cetuximab plus FOLFIRI, but *BRAF* tumor variant was a strong indicator of poor prognosis for all efficacy endpoints compared with those whose tumors were wild-type.

Clinically Useful

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

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 RCTs.
No RCTs were identified on the clinical utility of *BRAF* variant testing to predict nonresponse to anti-EGFR monoclonal antibody therapy.

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, based on clinical validity, cannot be constructed to support the use of the anti-EGFR monoclonal antibodies cetuximab and panitumumab for the treatment of patients with wild-type *BRAF* metastatic CRC.
Documentation of *KRAS* wild-type status is required prior to treatment with cetuximab or panitumumab. Documentation of *BRAF* variant status is not required but has been suggested to identify patients who are predicted to be nonresponders to anti-EGFR monoclonal antibody therapy.

Section Summary: *BRAF* Variant Testing to Guide Treatment for Metastatic Colorectal Cancer

Evidence for the clinical validity of *BRAF* variants in predicting nonresponse to anti-EGFR monoclonal antibody therapy includes 2 meta-analyses of prospective and retrospective analyses of RCTs. Subgroup analyses of *KRAS* wild-type and *NRAS* wild-type patients who did not respond to anti-EGFR monoclonal antibody therapy suggested that *BRAF* variants might be predictive of nonresponse. However, because of the low prevalence of *BRAF* variants, the true predictive value of *BRAF* variants is unclear. Direct evidence for the clinical validity of *BRAF* variant testing includes meta-analyses of prospective and retrospective analyses of RCTs. *BRAF* variant testing

has potential clinical utility in predicting nonresponse to anti-EGFR monoclonal antibody therapy in patients with documented *KRAS* wild-type and *NRAS* wild-type status. However, the direct evidence is limited for *BRAF* variant testing due to the low prevalence *BRAF* variants in CRC.

Microsatellite Instability High/Mismatch Repair Deficient Testing to Guide Treatment for Metastatic Colorectal Cancer

Clinical Context and Test Purpose

The purpose of Microsatellite-Instability/Mismatch Repair (MSI/MMR) testing in individuals with metastatic CRC is to guide decisions about treatment with immunotherapy.

The question addressed in this evidence review is: In individuals with metastatic CRC, does the use of MSI/MMR testing improve the net health outcome?

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

Populations

The relevant population of interest is individuals with metastatic CRC.

Interventions

The test being considered is MSI/MMR variant testing.

Comparators

The comparator of interest is standard treatment without MSI testing.

Outcomes

The beneficial outcomes of interest include PFS, OS, change in disease status, medication use, resource utilization, and treatment-related morbidity.

The time frame for outcome measures varies from several months to several years.

Study Selection Criteria

For the evaluation of clinical validity, 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;
- 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

Pembrolizumab

FDA Companion Diagnostic Test

There is currently no FDA-approved companion diagnostic test for the detection of high-frequency MSI (MSI-H)/deficient MMR (dMMR) status to select patients for treatment with pembrolizumab. The FDA approval of pembrolizumab in patients with metastatic CRC was based on results of KEYNOTE-177, a multicenter, randomized, open-label, active-controlled trial that enrolled 307 patients with previously untreated, unresectable or metastatic MSI-H or dMMR CRC. In the trial, MSI or MMR tumor status was determined locally using polymerase chain reaction or immunohistochemistry, respectively.

Randomized Controlled Trial

Evidence for the effectiveness of pembrolizumab in patients with MSI-H/dMMR metastatic CRC comes from the KEYNOTE-177 trial, reported by Andre et al (2020) (Tables 7-10).[42,]

The trial demonstrated a statistically significant improvement in PFS for patients randomized to pembrolizumab compared with chemotherapy (HR=0.60; 95% CI 0.45 to 0.80; p=.0002). At the time of the PFS analysis, the overall survival data were not mature (66% of the required number of events for the OS final analysis). The median follow-up time was 32.4 months (range: 24.0 to

48.3 months). The independent data monitoring committee recommended the continued masking of overall survival data until 190 deaths for the final analysis of overall survival have been observed or 12 months have elapsed since the last data review.

Table 7. Summary of Key RCT Characteristics

Study; Trial	Countries	Sites	Dates	Participants[2]	Interventions[1]	
					Active	Comparator
Andre et al (2020)[42,] KEYNOTE-177NCT02563002	23 countries	192	2016-2018	18 years of age or older with MSI-H/dMMR stage IV CRC	Pembrolizumab 200 mg every 3 weeks intravenously n = 153	Investigator's choice of chemotherapy determined within 3 days before randomization n = 154

CRC: colorectal cancer; MSH-H/dMMR: microsatellite instability-high/ mismatch repair deficient

Table 8. Summary of Key RCT Results

Study	Median Progression-free Survival, months (95% CI)	Patients Alive and Progression-free at 12 months	Patients Alive and Progression-free at 24 months	Response, % (95% CI)	Adverse Events
Andre et al (2020)[42,] KEYNOTE-177NCT02563002					
Pembrolizumab	16.5 (5.4 to 32.4)	55.3% (95% CI, 47.0 to 62.9)	48.3% (95% CI, 39.9 to 56.2)	Overall: 43.8% (35.8 to 52.0) Complete: 11% Duration: median not reached (range, 2.3+ to 41.4+)	Overall: 149/153 (97%) Grade 3 or higher: 86 (56%) Discontinuation due to adverse events:21 (14%)
Chemotherapy	8.2 (6.1 to 10.2)	37.3% (95% CI, 29.0 to 45.5)	18.6% (95% CI, 12.1 to 26.3)	Overall (complete or partial):33.1% (95% CI, 25.8 to 41.1) Complete: 4% Median duration: 0.6 months (range, 2.8 to 37.5+)	Overall:142/143 (99%) Grade 3 or higher:111 (78%) Discontinuation due to adverse events:17 (12%)
	Hazard Ratio=0.60				

	(95% CI 0.45 to 0.80); p =.0002				

CI: confidence interval

Table 9. Study Relevance Limitations

Study	Population[a]	Intervention[b]	Comparator[c]	Outcomes[d]	Duration of Follow-up[e]
Andre et al (2020)[42,] KEYNOTE-177NCT02563002				1. Overall survival not yet 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. Not clearly defined; 2. Version used unclear; 3. Delivery not similar intensity as comparator; 4.Not the intervention of interest.
[c] Comparator key: 1. Not clearly defined; 2. Not standard or optimal; 3. Delivery not similar intensity as intervention; 4. Not delivered effectively.
[d] Outcomes key: 1. Key health outcomes not addressed; 2. Physiologic measures, not validated surrogates; 3. No CONSORT reporting of harms; 4. Not establish and validated measurements; 5. Clinical significant difference not prespecified; 6. Clinical significant difference not supported.
[e] Follow-Up key: 1. Not sufficient duration for benefit; 2. Not sufficient duration for harms.

Table 10. Study Design and Conduct Limitations

Study	Allocation[a]	Blinding[b]	Selective Reporting[c]	Data Completeness[d]	Power[e]	Statistical[f]
Andre et al (2020)[42,] KEYNOTE-177NCT02563002		1, 2 open label				

The study limitations stated in this table are those notable in the current review; this is not a comprehensive gaps assessment.
[a] Allocation key: 1. Participants not randomly allocated; 2. Allocation not concealed; 3. Allocation concealment unclear; 4. Inadequate control for selection bias.
[b] Blinding key: 1. Not blinded to treatment assignment; 2. Not blinded outcome assessment; 3. Outcome assessed by treating physician.
[c] Selective Reporting key: 1. Not registered; 2. Evidence of selective reporting; 3. Evidence of selective publication.
[d] Data Completeness key: 1. High loss to follow-up or missing data; 2. Inadequate handling of missing data; 3. High number of crossovers; 4. Inadequate handling of crossovers; 5. Inappropriate exclusions; 6. Not intent to treat analysis (per protocol for noninferiority trials).
[e] Power key: 1. Power calculations not reported; 2. Power not calculated for primary outcome; 3. Power not based on clinically important difference.
[f] Statistical key: 1. Analysis is not appropriate for outcome type: (a) continuous; (b) binary; (c) time to event; 2. Analysis is not appropriate for multiple observations per patient; 3. Confidence intervals and/or p values not reported; 4. Comparative treatment effects not calculated.

Clinically Useful

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

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.

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.

Section Summary: Microsatellite Instability High/Mismatch Repair Deficient Testing to Guide Treatment for Metastatic Colorectal Cancer

Effectiveness of pembrolizumab compared to chemotherapy in patients with previously untreated, unresectable or metastatic MSI-H or dMMR CRC was investigated in a multicenter, randomized, open-label, active-controlled trial of 307 patients. The trial demonstrated a statistically significant improvement in PFS for patients randomized to pembrolizumab compared with chemotherapy.

Human Epidermal Growth Factor Receptor 2 Testing to Guide Treatment for Metastatic Colorectal Cancer

Clinical Context and Test Purpose

The purpose of human epidermal growth factor receptor 2 (HER2) testing in individuals with metastatic CRC is to determine HER2 status to inform decisions about targeted treatment.

The question addressed in this evidence review is: In individuals with metastatic CRC, does the use of HER2 testing improve the net health outcome in patients with metastatic CRC?

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

Populations

The relevant population of interest is individuals with metastatic CRC.

Interventions

The test being considered is HER2 testing. Use of HER2 testing is proposed to predict response to trastuzumab deruxtecan monotherapy or trastuzumab in combination with either pertuzumab or lapatinib.

Use of HER2 testing is also proposed to predict nonresponse to EGFR-targeted treatment.

Comparators

The following test strategy is currently being used: standard treatment with no HER2 testing.

Outcomes

The beneficial outcomes of interest include PFS, OS, change in disease status, medication use, resource utilization, and treatment-related morbidity.

The time frame for outcome measures varies from several months to several years.

Study Selection Criteria

For the evaluation of clinical validity, 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;
- 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

FDA Approved Companion Diagnostic Test

There is no FDA approved targeted treatment or companion diagnostic test for HER2 testing in patients with metastatic CRC. Multiple tests are approved for use to select targeted treatment.

Nonrandomized Trials

Hainsworth et al (2018) reported results of MyPathway, an open-label, phase 2, nonrandomized basket trial of targeted treatment in 251 patients with various advanced refractory solid tumors harboring genetic alterations.[43,] The cohort included 37 patients with HER2-ampified/overexpressed metastatic CRC. Treatment with trastuzumab plus pertuzumab produced partial response in 14 patients (38%; 95% CI, 23% to 55%) and the median duration of response was 11 months (range1 to 16+ months; 95% CI, 2.8 months to not estimable).
In an open-label, phase 2 trial of trastuzumab deruxtecan, objective response, the primary outcome, was observed in 24 of 53 patients with HER2-positive metastatic CRC (45.3%; 95% CI 31.6 to 59·6) after a median follow-up of 27.1 weeks (interquartile range [IQR] 19.3 to 40.1).[44,] One (2%) patient had a complete response, and 23 (43%) had a partial response. Median PFS was 6.9 months (4.1 to not evaluable). Median OS had not been reached at data cutoff (95% CI.74 months to not evaluable)
Preliminary evidence has suggested that HER2 amplification/overexpression may be predictive of nonresponse to EGFR-targeted therapy.[45,]

Clinically Useful

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

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.

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.

Section Summary: HER2 Testing to Guide Treatment for Metastatic Colorectal Cancer

There is no FDA-approved targeted treatment or companion diagnostic test for HER2 testing in patients with metastatic CRC. A phase 2 basket trial included 37 patients with HER2-ampified/overexpressed metastatic CRC. Treatment with trastuzumab plus pertuzumab produced partial response in 14 patients (38%; 95% CI, 23% to 55%) and the median duration of response was 11 months (range1 to 16+ months; 95% CI, 2.8 months to not estimable). In an open-label, phase 2 trial of trastuzumab deruxtecan, objective response was observed in 24 of 53 patients with HER2-positive metastatic CRC (45.3%; 95% CI 31.6 to 59.6) after a median follow-up of 27.1 weeks (IQR 19.3 to 40.1). Preliminary evidence has suggested that HER2 amplification/overexpression may be predictive of nonresponse to EGFR-targeted therapy.

Tumor Mutational Burden Testing to Guide Treatment for Metastatic Colorectal Cancer

Clinical Context and Test Purpose

The purpose of tumor mutational burden (TMB) testing in patients who have advanced CRC is to inform a decision on whether patients should receive immunotherapy versus another systemic therapy. The goal of immunotherapy is to preferentially kill malignant cells without significant damage to normal cells so that there is improved therapeutic efficacy along with decreased toxicity.
The question addressed in this evidence review is: In individuals with metastatic CRC, does the use of tumor mutational burden testing improve the net health outcome?
The following PICO was used to select literature to inform this review.

Populations

The relevant population of interest is individuals with metastatic CRC.

Interventions
The test being considered is TMB.
Tumor mutational burden, a measure of gene mutations within cancer cells, is proposed as a biomarker for response to immunotherapy.
Comparators
The following test strategy is currently being used: no TMB testing to guide treatment.
Outcomes
The beneficial outcomes of interest include PFS, OS, change in disease status, medication use, resource utilization, and treatment-related morbidity.
The time frame for outcome measures varies from several months to several years.
Study Selection Criteria
For the evaluation of clinical validity, 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;
- 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
FDA-Approved Companion Diagnostic Test
FoundationOne CDx is FDA approved as a companion diagnostic for use with pembrolizumab in patients with TMB-high (≥ 10 mutations per megabase) solid tumors. Approval was based on results of the KEYNOTE-158 study that enrolled patients with solid tumors, but none of the patients evaluated had CRC.
Nonrandomized Trial
Marabelle et al (2020) reported the association of high TMB to response to pembrolizumab in patients with solid tumors enrolled in a prespecified exploratory analysis of the KEYNOTE-158 study.[46,] High TMB was defined as >10 mutations per megabase according to the FoundationOne CDx panel. The proportion of patients with an objective response in the TMB-high group was 29%. At a median follow-up of approximately 3 years, the median duration of response was not reached in the TMB-high group and was 33.1 months in the non-TMB-high group. Notably, TMB-high status was associated with improved response irrespective of programmed death-ligand 1 (PD-L1). Median PFS and OS did not differ between the high and non-high TMB groups. Objective responses were observed in 24 (35%; 95% CI 24 to 48) of 68 participants who had both TMB-high status and PD-L1-positive tumors (ie, PD-L1 combined positive score of ≥1) and in 6 (21%; 8 to 40) of 29 participants who had TMB-high status and PD-L1-negative tumors. Study eligible cancers were limited to anal, biliary, cervical, endometrial, mesothelioma, neuroendocrine, salivary, small-cell lung, thyroid, and vulvar. Because no patients with colorectal cancer were included in these analyses, it is not possible to draw conclusions about the clinical validity and utility of TMB in this group of patients.
Clinically Useful
A test is clinically useful if the use of the results informs management decisions that improve the net health outcome of care. The net health outcome can be improved if patients receive correct therapy, or more effective therapy, or avoid unnecessary therapy, or avoid unnecessary testing.
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.

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.

Section Summary: Tumor Mutational Burden Testing to Guide Treatment for Metastatic Colorectal Cancer

In a prespecified retrospective subgroup analysis of a nonrandomized trial of pembrolizumab in patients with various solid tumors, objective responses were observed in 35% of participants who had both TMB-high status and PD-L1-positive tumors and in 21% of participants who had TMB-high status and PD-L1-negative tumors. A TMB-high status was associated with improved response irrespective of PD-L1 status. Median OS and PFS survival were not significantly different between TMB groups. Because no patients with CRC were included in these analyses, it is not possible to draw conclusions about the clinical validity and utility of TMB in this group of patients. These results need to be confirmed in well-designed prospective studies enrolling patients with CRC

Circulating Tumor DNA Testing (Liquid Biopsy) to Guide Treatment for Metastatic Colorectal Cancer

Clinical Context and Test Purpose

One purpose of liquid biopsy testing of patients who have metastatic CRC is to inform a decision regarding treatment selection (eg, whether to select a targeted treatment or standard treatment).

The question addressed in this evidence review is: Does use of circulating tumor DNA (ctDNA) or circulating tumor cells (CTCs) testing to select treatment in patients with metastatic CRC improve the net health outcome compared with standard tissue testing?

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

Populations

The relevant population of interest is individuals with metastatic CRC being considered for targeted therapy.

Interventions

The test being considered is liquid biopsy using either ctDNA or CTCs. Both targeted polymerase chain reaction-based assays and broad next-generation sequencing-based approaches are available.

Comparators

In patients who are able to undergo a biopsy, molecular characterization of the tumor is performed using standard tissue biopsy samples. Patients unable to undergo a biopsy generally receive standard therapy.

Outcomes

True-positive liquid biopsy test results lead to the initiation of appropriate treatment (eg, targeted therapy) without a tissue biopsy. False-positive liquid biopsy test results lead to the initiation of inappropriate therapy, which could shorten progression-free survival.

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

The time frame for outcomes measures varies from several months to several years.

Study Selection Criteria

For the evaluation of clinical validity, 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 (describe the 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

Given the breadth of molecular diagnostic methodologies available to assess ctDNA and CTC, the clinical validity of each commercially available test must be established independently. Multiple high-quality studies are needed to establish the clinical validity of a test.

OncoBEAM *RAS* CRC Assay

The clinical validity of the OncoBEAM *RAS* CRC assay has been evaluated in several published studies of patients with metastatic CRC. Study characteristics and results are shown in Tables 11 and 12. Study relevance, design, and conduct limitations are described in Tables 15 and 16.

Table 11. Clinical Validity Studies of the OncoBEAM *RAS* Assay

Study	Study Population	Design	Reference Standard	Timing of Tissue Biopsy and Liquid Biopsy	Blinding of Assessors
Garcia-Foncillas et al (2018)[47,]	• Patients with metastatic CRC newly diagnosed or presenting with recurrent disease after resection and/or chemotherapy at 10 centers in Spain • Enrolled from November 2015 to October 2016	Prospective	Analysis of tissue using standard-of-care procedures validated by each hospital	Plasma collected before any therapeutic intervention. OncoBEAM used when standard of care RAS result was discordant with RAS result. The same tissue block was used for re-analysis by OncoBEAM	Not stated; central laboratory used
Vidal et al (2017)[48,]	• Patients from Spain with histologically confirmed metastatic CRC • Anti-EGFR treatment-naive • Enrolled from 2009 to 2016	Retrospective-prospective	Analysis of tissue samples conducted using institutional standard-of-care procedures	• Tissue collected before blood • Median interval, 48 days (range, 0-1783 days)	Yes
Schmiegel (2017)[49,]	• Patients from Australia and Germany with newly diagnosed stage III/IV histologically confirmed CRC	Prospective	Analysis of tissue samples conducted using	• Blood obtained immediately prior to tissue biopsy or resection	Not stated

			Sanger sequencing		
Grasselli (2017)[50,]	• Patients from Spain with histologically confirmed metastatic CRC • Anti-EGFR treatment-naïve but majority treated with other systemic therapies	Retrospective-prospective	Analysis of tissue samples conducted using real-time PCR	Tissue collected before blood• Median interval 1.2 months (range 0 to 34)	Yes
Normanno (2018)[51,]	• Patients with metastatic CRC who are *KRAS* exon-2 wild-type and received first-line etuximab plus FOLFIRI within the CAPRI-GOIM trial	Retrospective-prospective	Analysis of tissue samples conducted using NGS	• Unclear when tissue was collected• Blood collected at baseline	Not stated

CRC: colorectal cancer; EGFR: epidermal growth factor receptor; FOLFIRI: folinic acid, fluorouracil, irinotecan; NGS: next-generation sequencing; PCR: polymerase chain reaction.

Table 12. Clinical Validity Studies of the OncoBEAM *RAS* Assay-Results

Study	Initial N	Final N	Excluded Samples	*RAS* Variant-Positive, %[a]	Sensitivity	Specificity	PPV	NPV
Garcia-Foncillas et al (2018)[47,]	239	236	3 patients initially excluded because of total disease removal during primary surgery. RAS mutation status was evaluable in all 236 patients	55.5	86.3	92.4	NR	NR
Vidal et al (2017)[48,]	NA	115	No description of samples excluded from comparison to tissue results	51	96 (87 to 100) [b]	90 (79 to 96) [b]	90 (79 to 96) [b]	96 (88 to 100) [b]
Schmiegel (2017)[49,]	102	98	n=3 (inadequate plasma DNA); n=1 (RAS mutation not confirmed in tissue when re-evaluated)	53	90 (79 to 96)	94(82 to 98)	NR	NR
Grasselli (2017)[50,]	157	146	N=11 (pre-analytical requirements or lack of tumor tissue availability)	59	89 (77 to 96)[b]	90 (82 to 95)[b]	84 (74 to 91)[b]	93 (87 to 97)[b]

Normanno (2018)[51,]	340	92	Tissue and plasma unavailable (not clear if tissue samples were sampled from those available or if all available were used)	36	70 (51 to 84)[b]	83 (71 to 92)[b]	70 (56 to 81)[b]	83 (74 to 89)[b]

CRC: colorectal cancer; NA: not available; NPV: negative predictive value; PPV: positive predictive value.
[a] With tissue biopsy reference standard.
[b] Values are percent with 95% confidence interval.
[b] Confidence intervals not reported in publication; calculated from data provided.

FoundationACT ctDNA Assay

The FoundationACT ctDNA assay, the predecessor of FoundationOne Liquid, was compared to tissue biopsy using the FoundationOne assay in one manufacturer-sponsored study by Li et al in 2019.[52,] Study characteristics and results are shown in Tables 13 and 14. The researchers reported results on the subset of 51 patients with *KRAS, NRAS,* and *BRAF* variants. These results are shown in Table 10. Positive percent agreement was 80% for all time points for short variants and increased to 90% for cases in which tissue and liquid biopsy were measured less than 270 days apart. Limitations of this study are described in Tables 15 and 16.

Table 13. Clinical Validity Study of the FoundationACT ctDNA Assay

Study	Study Population	Design	Reference Standard	Timing of Reference and Index Tests	Blinding of Assessors
Li et al (2019)[52,]	Patients with CRC, 74% stage IV, 19% stage III, 7% stage II	Prospective and retrospective	Previously-collected tissue biopsy with FoundationOne assay	Liquid biopsy testing was done at the discretion of the clinician at variable time intervals after tissue sample collection (0–709 days).	Not stated

CRC: colorectal cancer; ctDNA: circulating tumor DNA.

Table 14. Clinical Validity Study of the FoundationACT ctDNA Assay - Results

Study	Initial N	Final N	Excluded Samples	*RAS* Variant-Positive, %	Positive Percent Agreement (95% Confidence Interval)
Li et al (2019)[52,]	96	73	22 samples did not have detectable ctDNA	51/74 (92%)	Overall (N=73): 79% Subset with *KRAS*, *NRAS*, and *BRAF* variants (n=51): 80% for all timepoints 90% for cases <270 days between tissue and liquid biopsy

ctDNA: circulating tumor DNA.; PPV: positive predictive value.

Table 15. Study Relevance Limitations for Clinical Validity Studies of Liquid Biopsy in Metastatic Colorectal Cancer

Study	Population[a]	Intervention[b]	Comparator[c]	Outcomes[d]	Duration of Follow-Up[e]

Li et al (2019)[52,]	4.74% had metastatic disease		2. Reference standard was FoundationOne assay		
Garcia-Foncillas et al (2018)[47,]				3. PPV and NPV not reported	
Vidal et al (2017)[48,]					
Schmiegel (2017)[49,]		2: Not clear if marketed version of test used			
Grasselli (2017)[50,]					
Normanno (2018)[51,]					

The study limitations stated in this table are those notable in the current review; this is not a comprehensive gaps assessment.
NPV: negative predictive value; PPV: positive predictive value.
[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 for Liquid Biopsy in Metastatic Colorectal Cancer

Study	Selection[a]	Blinding[b]	Delivery of Test[c]	Selective Reporting[d]	Completeness of Follow-Up[e]	Statistical[f]
Li et al (2019)[52,]	2. Inclusion required a previously performed FoundationACT assay; previous treatments varied	1: blinding unclear	2. timing of liquid biopsy and tissue biopsy varied (range 0-709 days)		2. 20% of samples had no detectable ctDNA	
Garcia-Foncillas et al (2018)[47,]	1. Not clear whether samples were consecutive or convenience	1: blinding unclear		1. Registration not described		
Vidal et al (2017)[48,]	1. Not clear whether		2: Blood collected	1. Registration	1. Not clear whether there	1. CIs not reported but

	samples were consecutive or convenience		approximately 1.5 m after tissue	not described	were samples that were insufficient for analysis or failed to produce results	calculated based on data provided
Schmiegel (2017)[49],	1: Not clear how patients were selected from those that were eligible	1: Blinding unclear		1. Registration not described		
Grasselli (2017)[50],	1: Not clear how patients were selected from those that were eligible		2: Blood collected approximately 1.5 m after tissue			1. CIs not reported but calculated based on data provided
Normanno (2018)[51],	1: Not clear how tumor samples were selected from those available	1: Blinding unclear	1: Unclear when tissue was collected	1. Registration not described	2: Only 27% of CAPRI-GOIM trial participants included	1. CIs not reported but calculated based on data provided

The study limitations stated in this table are those notable in the current review; this is not a comprehensive gaps assessment.
CI: confidence interval; 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] Follow-Up key: 1. Inadequate description of indeterminate and missing samples; 2. High number of samples/patients 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, or more effective therapy, or avoid unnecessary therapy, or avoid unnecessary testing.

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 RCTs.

No RCTs were identified on the clinical utility of liquid biopsy to guide treatment for patients with metastatic CRC.

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.

Section Summary: Circulating Tumor DNA Testing (Liquid Biopsy) to Guide Treatment for Metastatic Colorectal Cancer

The clinical validity of the OncoBEAM RAS CRC Assay has been studied in multiple observational studies. When compared to tissue biopsy, sensitivity ranged from 70% (51% to 84%) to 96% (95% CI 87% to 100%) and specificity ranged from 83% (95% CI 71% to 92%) to 94% (82% to 98%). FoundationOne Liquid has been compared to tissue biopsy with the FoundationACT

assay in 1 observational study; positive percent agreement was 80% overall and 90% when tissue and liquid biopsy were collected less than 270 days apart. Clinical validity studies were limited by unclear reporting of blinding, use of convenience rather than consecutive samples, and variation in the timing of sample collection. There are no published studies reporting clinical outcomes or clinical utility.

Summary of Evidence

For individuals with metastatic CRC who receive *KRAS* variant testing to guide treatment, the evidence includes multiple systematic reviews including a TEC Assessment. Relevant outcomes are OS, disease-specific survival, change in disease status, medication use, resource utilization, and treatment-related morbidity. Variant testing of tumor tissue performed in prospective and retrospective analyses of randomized controlled trials has consistently shown that the presence of a *KRAS* variant predicts nonresponse to cetuximab and panitumumab, either as monotherapy or in combination with other treatment regimens. Analyses also support the use of *KRAS* variant analysis of tumor DNA before considering a treatment regimen. The evidence is sufficient to determine that the technology results in an improvement in the net health outcome.

For individuals with metastatic CRC who receive *NRAS* variant testing to guide treatment, the evidence includes prospective-retrospective analyses of randomized controlled trials and retrospective cohort studies. Relevant outcomes are OS, disease-specific survival, change in disease status, medication use, resource utilization, and treatment-related morbidity. Pooled analyses have shown that *NRAS* variants (beyond the common *KRAS* exon 2 variants) predict nonresponse to cetuximab and panitumumab and support the use of *NRAS* variant analysis of tumor DNA before considering a treatment regimen. In addition, there is strong support from the National Comprehensive Cancer Network and the American Society of Clinical Oncology for *NRAS* and *KRAS* testing in patients with metastatic CRC. The evidence is sufficient to determine that the technology results in an improvement in the net health outcome.

For individuals with metastatic CRC who receive *BRAF* variant testing to guide treatment, the evidence includes 2 meta-analyses of prospective and retrospective analyses of randomized controlled trials. Relevant outcomes are OS, disease-specific survival, change in disease status, medication use, resource utilization, and treatment-related morbidity. The meta-analyses have shown that anti-EGFR monoclonal antibody therapy did not improve survival in patients with *RAS* wild-type or *BRAF*-mutated tumors; however, the individual studies have been small, and the results have been inconsistent. The evidence is insufficient to determine that the technology results in an improvement in the net health outcome.

For individuals with metastatic CRC who receive MSI/MMR testing to guide treatment, the evidence includes an RCT of pembrolizumab compared to chemotherapy and nonrandomized trials. Relevant outcomes are OS, disease-specific survival, change in disease status, medication use, resource utilization, and treatment-related morbidity. Effectiveness of pembrolizumab compared to chemotherapy in patients with previously untreated, unresectable or metastatic MSI-H or dMMR CRC was investigated in a multicenter, randomized, open-label, active-controlled trial of 307 patients. The trial demonstrated a statistically significant improvement in progression free survival for patients randomized to pembrolizumab compared with chemotherapy (HR 0.60; 95% confidence interval [CI] 0.45 to 0.80; p=.0002). The evidence is sufficient to determine that the technology results in an improvement in the net health outcome.

For individuals with metastatic CRC who receive HER2 testing to guide treatment, the evidence includes nonrandomized trials. Relevant outcomes are OS, disease-specific survival, change in disease status, medication use, resource utilization, and treatment-related morbidity. There is no approved targeted treatment or companion diagnostic test for HER2 testing in patients with metastatic CRC. A phase 2 basket trial included 37 patients with HER2-ampified/overexpressed

metastatic CRC. Treatment with trastuzumab plus pertuzumab produced partial response in 14 patients (38%; 95% CI, 23% to 55%) and the median duration of response was 11 months (range1 to 16+ months; 95% CI, 2.8 months to not estimable). In an open-label, phase 2 trial of trastuzumab deruxtecan, objective response was observed in 24 of 53 patients with HER2-positive metastatic CRC (45.3%; 95% CI 31.6 to 59.6) after a median follow-up of 27.1 weeks (IQR 19.3 to 40.1). Preliminary evidence has suggested that patients with HER2-amplified metastatic CRC are less likely to respond to anti-EGFR therapy. The evidence is insufficient to determine that the technology results in an improvement in the net health outcome.

For individuals with metastatic CRC who receive TMB testing to select treatment with immunotherapy, the evidence includes a prespecified retrospective subgroup analysis of a nonrandomized phase 2 trial. Relevant outcomes are OS, disease-specific survival, and test accuracy. Objective responses were observed in 35% of participants who had both TMB-high status and PD-L1-positive tumors and in 21% of participants who had TMB-high status and PD-L1-negative tumors. High TMB status was associated with improved response irrespective of PD-L1 status. Median OS and progression free survival were not significantly different between TMB groups. Because no patients with CRC were included in these analyses, it is not possible to draw conclusions about the clinical validity and utility of TMB in this group of patients. Well-designed prospective studies enrolling patients in the population of interest are required. The evidence is insufficient to determine that the technology results in an improvement in the net health outcome.

For individuals with metastatic CRC who receive circulating tumor DNA or circulating tumor cell testing (liquid biopsy) to guide treatment, the evidence includes observational studies. Relevant outcomes are OS, disease-specific survival, test validity, morbid events, and medication use. Given the breadth of methodologies available to assess circulating tumor DNA and circulating tumor cells, the clinical validity of each commercially available test must be established independently. The clinical validity of the OncoBEAM™ RAS CRC Assay has been studied in multiple observational studies. When compared to tissue biopsy, sensitivity ranged from 70% (51% to 84%) to 96% (95% CI 87% to 100%) and specificity ranged from 83% (95% CI 71% to 92%) to 94% (82% to 98%). FoundationOne® Liquid has been compared to tissue biopsy with the FoundationACT™ assay in 1 observational study; positive percent agreement was 80% overall and 90% when tissue and liquid biopsy were collected less than 270 days apart. Clinical validity studies were limited by unclear reporting of blinding, use of convenience rather than consecutive samples, and variation in the timing of sample collection. There are no published studies reporting clinical outcomes or clinical utility. 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.

Clinical Input From Physician Specialty Societies and Academic Medical Centers

While the various physician specialty societies and academic medical centers may collaborate with and make recommendations during this process, through the provision of appropriate reviewers, input received does not represent an endorsement or position statement by the physician specialty societies or academic medical centers, unless otherwise noted.

2017 Input

Clinical input was sought to help determine whether the use of *BRAF* V600E variant analysis for individuals with metastatic CRC who are found to be wild-type on *KRAS* and *NRAS* variant analysis provides a clinically meaningful improvement in net health outcome and is consistent with generally accepted medical practice. In response to requests, clinical input was received

from 10 respondents, including 2 specialty society-level responses, 1 physician from an academic center, and 6 physicians from 2 health systems.
For individuals who have metastatic CRC who are found to be wild-type on *KRAS* and *NRAS* variant analysis who receive *BRAF* V600E variant analysis to guide management decisions, clinical input supports this use provides a clinically meaningful improvement in net health outcome and indicates this use is consistent with generally accepted medical practice.

Practice Guidelines and Position Statements

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

American Society of Clinical Oncology et al

In 2017, the American Society of Clinical Oncology along with American Society for Clinical Pathology, College of American Pathologists, and Association for Molecular Pathology published guidelines on molecular biomarkers for the evaluation of colorectal cancer.[53,] Table 17 summarizes the relevant guidelines.

Table 17. Summary of Recommendations

Guidelines	Type	SOE	QOE
Colorectal carcinoma patients being considered for anti-EGFR therapy must receive RAS mutational testing. Mutational analysis should include KRAS and NRAS codons 12, 13 of exon 2; 59, 61 of exon 3; and 117 and 146 of exon 4 ("expanded" or "extended" RAS)	Recommendation	Convincing/ adequate, benefits outweigh harms	High/intermediate
BRAF p.V600 (BRAF c. 1799 (p.V600) mutational analysis should be performed in colorectal cancer tissue in patients with colorectal carcinoma for prognostic stratification	Recommendation	Adequate/inadequate, balance of benefits and harms	Intermediate/low
BRAF p.V600 mutational analysis should be performed in deficient MMR tumors with loss of MLH1 to evaluate for Lynch Syndrome risk. Presence of a BRAF mutation strongly favors sporadic pathogenesis. The absence of BRAF mutation does not exclude risk of Lynch syndrome	Recommendation	Adequate/inadequate, balance of benefits and harms	Intermediate/low
Clinicians should order mismatch repair status testing in patients with colorectal cancers for the identification of patients at high-risk for Lynch syndrome and/or prognostic stratification	Recommendation	Adequate/inadequate, balance of benefits and harms	Intermediate/low
There is insufficient evidence to recommend BRAF c.1799 p.V600 mutational status as a	No recommendation	Insufficient, benefits/harms balance unknown	Insufficient

Guidelines	Type	SOE	QOE
predictive molecular biomarker for response to anti-EGFR inhibitors			

EGFR: epidermal growth factor receptor; QOE: quality of evidence; SOE: strength of evidence.

In 2015, the American Society of Clinical Oncology updated its provisional clinical opinion on extended *RAS* variant testing in metastatic colorectal cancer to predict response to anti-epidermal growth factor receptor (EGFR) monoclonal antibody therapy.[54,] The opinion was based on evidence from 13 articles on *KRAS* variants (11 systematic reviews, 2 health technology assessments) and 2 articles on *NRAS* testing. The opinion stated that subgroup analyses of patients with any of the less common *RAS* variants were small, and there was inadequate evidence to provide a definitive opinion on the lack of benefit for the use of anti-epidermal growth factor receptor antibodies for patients whose cancer harbors any specific *RAS* variant other than the exon 2 *KRAS* variant. The Society considered the less common *RAS* variants as a group, and a pooled analysis suggested the same lack of benefit with anti-epidermal growth factor receptor therapy as seen with the more common variants in exon 2 of *KRAS*.

National Comprehensive Cancer Network

RAS and BRAF Testing

The National Comprehensive Cancer Network (NCCN) guidelines on the treatment of colon cancer (v.2.2021) recommend that tumor tissue should be genotyped for RAS (KRAS and NRAS) and BRAF variants, individually or as part of a next-generation sequencing panel, for all patients with metastatic colon cancer.[55,] Patients with any known KRAS mutation (exon 2, 3, 4) or NRAS mutation (exon 2, 3, 4) should not be treated with either cetuximab or panitumumab. BRAF V600E mutation makes response to panitumumab or cetuximab highly unlikely unless given with a BRAF inhibitor (Category 2A).

Microsatellite Instability/Mismatch Repair Testing

The guidelines recommend universal mismatch repair (MMR) or microsatellite instability (MSI) testing for all patients with a personal history of colon or rectal cancer. In addition to its role as a predictive marker for immunotherapy use in the advanced colorectal cancer setting, MMR/MSI status can also help to identify individuals with Lynch syndrome and to inform adjuvant therapy decisions for patients with stage II disease (Category 2A).

Human Epidermal Receptor 2 Testing

The guidelines recommend testing for human epidermal receptor 2 (HER2) amplifications for patients with metastatic colorectal cancer. Anti-HER2 therapy is only indicated in HER2-ampliified tumors that are also RAS and BRAF wild type. If the tumor is already known to have a *KRAS/NRAS* or *BRAF* mutation, HER2 testing is not indicated. As HER2-targeted therapies are still under investigation, enrollment in a clinical trial is encouraged (Category 2A).

Tumor Mutational Burden Testing

Based on the limited data in the colorectal cancer population, the NCCN Panel does not currently recommend tumor mutational burden biomarker testing, unless measured as part of a clinical trial.

Circulating Tumor DNA

The NCCN Panel states there are insufficient data to recommend the use of multigene assays, Immunoscore, or post-surgical circulating tumor DNA to estimate risk recurrence or determine adjuvant therapy.

U.S. Preventive Services Task Force Recommendations

Not applicable.

Ongoing and Unpublished Clinical Trials

Currently unpublished trials that might influence this review are listed in Table 18.

Table 18. Summary of Key Ongoing Trials

NCT No.	Trial Name	Planned Enrollment	Completion Date
Ongoing			
NCT03038217	Investigation of the Value of ctDNA Analysis in the Diagnosis, Treatment, and Surveillance of Patients With Surgically Resectable Colorectal Cancer	300	Dec 2021
NCT04425239	Intermittent or Continuous Panitumumab Plus FOLFIRI for First-line Treatment of Patients With RAS/B-RAF Wild-type Metastatic Colorectal Cancer: a Randomized Phase 2 Trial	136	Dec 2021
NCT04008030	A Phase 3 Randomized Clinical Trial of Nivolumab Alone, Nivolumab in Combination With Ipilimumab, or an Investigator's Choice Chemotherapy in Participants With Microsatellite Instability High (MSI-H) or Mismatch Repair Deficient (dMMR) Metastatic Colorectal Cancer	748	Aug 2025
NCT02563002	A Phase III Study of Pembrolizumab (MK-3475) vs. Chemotherapy in Microsatellite Instability-High (MSI-H) or Mismatch Repair Deficient (dMMR) Stage IV Colorectal Carcinoma (KEYNOTE-177)	307	Feb 2023
NCT02997228	Colorectal Cancer Metastatic dMMR/MSI-H Immuno-Therapy (COMMIT) Study: A Randomized Phase III Study of mFOLFOX6/Bevacizumab/Atezolizumab Combination Versus Single Agent Atezolizumab in the First-Line Treatment of Patients With Deficient DNA Mismatch Repair (dMMR)/Microsatellite Instability-High (MSI-H) Metastatic Colorectal Cancer	231	Apr 2022
NCT03365882	S1613, A Randomized Phase II Study of Trastuzumab and Pertuzumab (TP) Compared to Cetuximab and Irinotecan (CETIRI) in Advanced/Metastatic Colorectal Cancer (mCRC) With HER-2 Amplification	130	Jun 2023
NCT02465060	Targeted Therapy Directed by Genetic Testing in Treating Patients With Advanced Refractory Solid Tumors, Lymphomas, or Multiple Myeloma (The MATCH Screening Trial)	6452	Jun 2022
NCT03602079	A Phase I-II, FIH Study of A166 in Locally Advanced/Metastatic Solid Tumors Expressing Human Epidermal Growth Factor Receptor 2 (HER2) or Are HER2 Amplified That Did Not Respond or Stopped Responding to Approved Therapies	82	Dec 2021

NCT: national clinical 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

81210	BRAF (B-Raf proto-oncogene, serine/threonine kinase) (eg, colon cancer, melanoma), gene analysis, V600 variant(s)
81275	KRAS (Kirsten rat sarcoma viral oncogene homolog) (eg, carcinoma) gene analysis; variants in exon 2 (eg, codons 12 and 13)
81276	KRAS (Kirsten rat sarcoma viral oncogene homolog) (eg, carcinoma) gene analysis; additional variant(s) (eg, codon 61, codon 146)
81301	Microsatellite instability analysis (eg, hereditary non-polyposis colorectal cancer, Lynch syndrome) of markers for mismatch repair deficiency (eg, BAT25, BAT26), includes comparison of neoplastic and normal tissue, if performed
81311	NRAS (neuroblastoma RAS viral [v-ras] oncogene homolog) (eg, colorectal carcinoma), gene analysis, variants in exon 2 (eg, codons 12 and 13) and exon 3 (eg, codon 61)
88363	Examination and selection of retrieved archival (ie, previously diagnosed) tissue(s) for molecular analysis (eg, KRAS mutational analysis)
0111U	Oncology (colon cancer), targeted KRAS (codons 12, 13 and 61) and NRAS (codons 12, 13 and 61) gene analysis utilizing formalin-fixed paraffin-embedded tissue
0242U	Targeted genomic sequence analysis panel, solid organ neoplasm, cell free circulating DNA analysis of 55-74 genes, interrogation for sequence variants, gene copy number amplifications, and gene rearrangements

ICD-10 Diagnoses

C18.0	Malignant neoplasm of cecum
C18.1	Malignant neoplasm of appendix
C18.2	Malignant neoplasm of ascending colon
C18.3	Malignant neoplasm of hepatic flexure
C18.4	Malignant neoplasm of transverse colon
C18.5	Malignant neoplasm of splenic flexure
C18.6	Malignant neoplasm of descending colon
C18.7	Malignant neoplasm of sigmoid colon
C18.8	Malignant neoplasm of overlapping sites of colon
C19	Malignant neoplasm of rectosigmoid junction
C20	Malignant neoplasm of rectum
C78.5	Secondary malignant neoplasm of large intestine and rectum

REVISIONS

07-10-2015	Policy added to the bcbsks.com web site on 06-10-2015 with an effective date of 07-10-2015.
01-01-2016	In Coding section: ▪ Added CPT codes: 81276, 81311. ▪ Revised nomenclature of codes: 81210, 81275.
08-29-2016	Updated Description section.
	In Policy section: ▪ In Item B, removed "experimental / investigational", "to", "and", and "in the treatment of metastatic colorectal cancer" and added "medically necessary", "for patients with", "prior to planned therapy with", and "or" to read "*NRAS* mutation analysis is considered medically necessary for patients with metastatic colorectal cancer to predict nonresponse prior to planned therapy with anti-EGFR monoclonal antibodies cetuximab or panitumumab."
	Updated Rationale section.
	In Coding section: ▪ Removed CPT codes: 81403, 81404.
	Updated References section.
01-30-2018	Updated Policy title from "KRAS, NRAS, and BRAF Mutation Analysis in Metastatic Colorectal Cancer" to "KRAS, NRAS, and BRAF Variant Analysis in Metastatic Colorectal Cancer."
	Updated Description section.
	In Policy section: ▪ In Item A, removed "mutation" and added "variant" and "epidermal growth factor" to read, "*KRAS* variant analysis may be considered medically necessary for patients with metastatic colorectal cancer to predict nonresponse prior to planned therapy with anti-epidermal growth factor (EGFR) monoclonal antibodies cetuximab or panitumumab." ▪ In Item B, removed "mutation" and "is" and added "variant" and "may be" to read, "*NRAS* variant analysis may be considered medically necessary for patients with metastatic colorectal cancer to predict nonresponse prior to planned therapy with anti-EGFR monoclonal antibodies cetuximab or panitumumab. ▪ In Item C, removed "mutation", "is", "experimental/investigational", and "to predict nonresponse to anti-EGFR monoclonal antibodies cetuximab and panitumumab in the treatment of metastatic colorectal cancer" to read, "*BRAF* variant analysis may be is considered medically necessary for patients with metastatic colorectal cancer who are found to be wild-type on *KRAS* and *NRAS* variant analysis to guide management decisions." ▪ Added Policy Guidelines.
	Updated Rationale section.
	In Coding section: ▪ Removed ICD-9 codes.
	Updated References section.
	Added Appendix section.
08-29-2018	Updated Description section.
	Updated Rationale section.
	Updated References section.
	Removed Appendix.
09-27-2019	Policy published to the bcbsks.com website on 08-28-2019 with an effective date of 09-27-2019.
	Updated Description section.
	In Policy section:

	▪ Added new Item D, "*KRAS, NRAS,* and *BRAF* variant analysis using circulating tumor DNA or circulating tumor cell testing (liquid biopsy) to guide treatment for patients with metastatic colorectal cancer is considered experimental / investigational."
	Updated Rationale section.
	In Coding section: ▪ Added CPT codes: 86152, 86153, 0069U. ▪ Removed coding bullets.
	Updated References section.
10-01-2019	In Coding section: ▪ Added PLA code: 0111U
04-30-2021	Updated Description section
	Updated Rationale section
	In Coding section: • Removed CPT codes 86152 and 86153 • Added CPT code 0242U
	Updated References section
	Added Appendix 1 and 2
10-10-2021	Changed Title from "KRAS, NRAS, and BRAF Variant Analysis in Metastatic Colorectal Cancer" to "Biomarker Testing (Including Liquid Biopsy) for Targeted Treatment and Immunotherapy in Metastatic Colorectal Cancer"
	Updated Description section.
	In Policy section: ▪ Added Items C, D, and F. ▪ Added "as well as Mismatch repair/microsatellite instability (MMR/MSI) testing," to Item G
	Updated Rationale section.
	In Coding section: ▪ Added code 81301 ▪ Removed code 0069U
	Updated References section.

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APPENDIX

1 - APPENDIX 1: CLINICAL INPUT

Appendix Table 1. Respondent Profile

	Specialty Society				
No	**Name of Organization**			**Clinical Specialty**	
1	Association for Molecular Pathology			Molecular Pathology	
	Physician				
No	**Name**	**Degree**	**Institutional Affiliation**	**Clinical Specialty**	**Board Certification and Fellowship Training**
Identified by Cancer Treatment Centers of America					
2	Anonymous	MD	Cancer Treatment Centers of America (CTCA)	Medical oncologist	Internal Medicine and Medical Oncology
3	Eyal Meiri	MD	Cancer Treatment Centers of America (CTCA)	Medical oncology	Medical Oncology
4	Arturo Loaiza-Bonilla	MD, MS Ed	Cancer Treatment Centers of America (CTCA)	Medical oncology, gastrointestinal oncology	ABIM certified in Internal Medicine, Medical Oncology and Hematology. Fellowship.
5	Anonymous	MD	Cancer Treatment Centers of America (CTCA)	Pathology and laboratory medicine	American Board of Pathology
6	Shahin Chowdhury	DO	Cancer Treatment Centers of America (SERMC)	Medical oncology	American College of Osteopathic Internists
Identified by Dan L Duncan Comprehensive Cancer Center, Baylor College of Medicine					
7	Brandon G. SmagloManisha Chandar	MDDO	Dan L Duncan Comprehensive Cancer Center, Baylor College of Medicine	BGS: Gastrointestinal oncology MC: Hematology/oncology	BGS: Boarded in Medical Oncology and Internal Medicine. Fellowship training at Georgetown Lombardi Comprehensive Cancer Center, 2013. MC: Boarded in Internal Medicine.

					Current second year fellow, Baylor.
Identified by American Society of Clinical Oncology					
8	Carmen J. Allegra	MD	University of Florida	Medical oncology	Internal Medicine and Oncology
9	Christopher H. Lieu	MD	University of Colorado	Medical oncology	Medical Oncology - Fellowship Training - MD Anderson Cancer Center
Identified by Catholic Health Initiatives					
10	Anonymous	MD		Medical oncology	Medical Oncology and Internal Medicine

Appendix Table 2. Respondent Conflict of Interest Disclosure

No.	**1. Research support related to the topic where clinical input is being sought**		**2. Positions, paid or unpaid, related to the topic where clinical input is being sought**		**3. Reportable, more than $1000, healthcare-related assets or sources of income for myself, my spouse, or my dependent children related to the topic where clinical input is being sought**		**4. Reportable, more than $350, gifts or travel reimbursements for myself, my spouse, or my dependent children related to the topic where clinical input is being sought**	
	Yes/No	Explanation	Yes/No	Explanation	Yes/No	Explanation	Yes/No	Explanation
2	No		No		No		No	
3	No		No		No		No	
4	No		No		No		No	
5	No		No		No		No	
6	No		No		No		No	
7	No		Yes (BGS) No (MC)	BGS: Speaker's bureau for TAIHO oncology for the colorectal cancer drug Lonsurf	No		No	
8	No		Yes	ASCO representative for colorectal cancer biomarkers	No		No	

No.	1. Research support related to the topic where clinical input is being sought		2. Positions, paid or unpaid, related to the topic where clinical input is being sought		3. Reportable, more than $1000, healthcare-related assets or sources of income for myself, my spouse, or my dependent children related to the topic where clinical input is being sought		4. Reportable, more than $350, gifts or travel reimbursements for myself, my spouse, or my dependent children related to the topic where clinical input is being sought	
	Yes/No	Explanation	Yes/No	Explanation	Yes/No	Explanation	Yes/No	Explanation
9	No		No		No		No	
10	No		No		No		No	
No.	Conflict of Interest Policy Statement							
1	No conflict of interest							

Individual physician respondents answered at individual level. Specialty Society respondents provided aggregate information that may be relevant to the group of clinicians who provided input to the Society-level response.
NR: not reported.

2 - APPENDIX 2: CLINICAL INPUT RESPONSES

CI - Objective

The epidermal growth factor receptor (EGFR) is overexpressed in colorectal cancer (CRC). *EGFR*-targeted therapy, with monoclonal antibodies cetuximab and panitumumab, has shown a clear survival benefit in patients with metastatic CRC. However, this benefit depends on a lack of variants in certain genes in the signaling pathway downstream from the *EGFR*. It has been hypothesized that knowledge of tumor cell *KRAS*, *NRAS*, and *BRAF* variant status might be used as a predictor of nonresponse to anti-EGFR monoclonal antibody therapy.

The following PICO applies to this indication.

Populations	Interventions	Comparators	Outcomes
Individuals: • With metastatic colorectal cancer	**Interventions of interest are:** • *BRAF* variant testing to guide treatment	**Comparators of interest are:** • No *BRAF* variant testing to guide treatment	**Relevant outcomes include:** • Overall survival • Disease-specific survival • Change in disease status • Medication use • Treatment-related morbidity

Clinical input is sought to help determine whether testing for *BRAF* V600E variant status for individuals with metastatic CRC would provide a meaningful clinical benefit, defined as avoidance

of anti-EGFR targeted therapies that are unlikely to result in an objective tumor response in patients, and whether this use is consistent with generally accepted medical practice.

Responses

- Based on the evidence and your clinical experience, please describe the clinical context that may offer clinical benefit associated with testing for *BRAF* V600E variant status for individuals with metastatic CRC to guide treatment with *EGFR*-targeted therapy. Please comment on what predictive value of testing for *BRAF* V600E variant status would be needed for a clinically meaningful benefit from avoiding anti-EGFR targeted therapies. Also include any sequencing considerations with other evaluation and testing. Please include supporting rationale and relevant references to support your clinical input.

No.	Response
1	In March 2017, the American Society for Clinical Pathology (ASCP), College of American Pathologists (CAP), Association for Molecular Pathology (AMP), and American Society of Clinical Oncology (ASCO) published an updated guideline on Molecular Biomarkers for the Evaluation of Colorectal Cancer. This is an evidence-based guideline recommendation, which was constructed through a systematic review of the literature to establish standard molecular biomarker testing of CRC tissue to guide EGFR therapies and conventional chemotherapy regimens. We recommend review and incorporation of these guidelines into your evidence review and summaries for colorectal cancer. Our comments in this clinical input reflect recommendations within the guideline. The guideline supports extended RAS testing along with the following recommendations: • While BRAF status does not directly inform about response to anti EGFR therapies, it is a poor prognostic indicator in high stage cancers and has important value generally in informing therapeutic decision making for those patients. Specifically, the ASCP/CAP/AMP/ASCO guideline states that BRAF V600 position mutational status is recommended for prognostic stratification in selected patients with CRC (Recommendation 2a) and that there is insufficient evidence to recommend BRAF pV600 mutational status as a predictive molecular biomarker for response to anti-EGFR inhibitors (Recommendation 4). Briefly, the guidelines state: "BRAF activating mutations occur in about 8% of advanced disease patients with CRC and in approximately 14% of patients with localized stage II and III CRC. As such, mutations in BRAF constitute a substantial subset of patients with CRC. Four systematic reviews and three systematic reviews that included meta-analyses pertaining to the prognostic and predictive value of BRAF mutations in patients with CRC were identified through our systematic review process. These studies revealed that patients with advanced CRC who possess a BRAF mutation have significantly poorer outcomes as measured by PFS and OS and have a decreased response rate to anti-EGFR therapy relative to those with nonmutated BRAF. Poorer OS was also demonstrated for those patients with earlier stage II and III CRC having a BRAF mutation; however, the poorer outcome appears to be primarily the result of decreased OS after relapse in these patients rather than a harbinger of an increased rate of relapse. Finally, while outcomes in advanced disease patients with BRAF mutations were poorer relative to nonmutation patients, the data were consistent with a modest beneficial impact from the use of anti-EGFR agents relative to those patients whose tumors contained a RAS mutation. In summary, patients with CRC that contains a BRAF mutation have a worse outcome relative to nonmutation patients. Selected patients for BRAF mutation testing include patients with metastatic disease, since these patients have particularly poor outcomes. It is important to know the BRAF c.1799 (p.V600) mutation status of a patient's CRC since standard therapy is not adequate for patients with metastatic disease and BRAF mutation. For these patients, some studies suggest the use of FOLFIRINOX [folinic

No.	Response
	acid (leucovorin calcium), 5-fluorouracil, irinotecan hydrochloride, and oxaliplatin] as first-line therapy, followed by enrollment in a clinical trial." • Further, clinicians should order mismatch repair status testing in patients with colorectal cancers for the identification of patients at high risk for Lynch syndrome and/or prognostic stratification (Recommendation 3), a recommendation which is supported in the BCBSKS medical policy "Genetic Testing for Lynch Syndrome and Other Inherited Colon Cancer Syndromes."
2	BRAF V600E occurs in less than 10% of sporadic colorectal carcinoma. There is a strong negative prognostic marker for both early and late-stage colorectal carcinoma especially in the non-MSI-H tumors. MSI-H tumors which have BRAF mutation may not have the same adverse prognostic responses. BRAF V 600E mutations showed resistance to anti EGFR therapy.
3	Pooled analysis and meta-analysis presented in summary report is self-explanatory. BRAF V600E mutations are a predictor of poor response to anti-EGFR therapy and in general represent a poor prognostic category of patients. Upon testing for RAS variants, should no mutations for RAS be found, BRAF mutations analysis should be obtained.
4	Overall, the presence of BRAF mutation is an indicator of poor prognosis and a potential target for clinical trials, currently testing combinations of BRAF inhibitor, MEK inhibitor and EGFR inhibitor. As such, the only clinical benefit of testing BRAF variant to guide treatment would be to consider an earlier introduction of clinical trial with combination targeted therapy, given the poor prognosis of these patients.Only one meta-analysis provided evidence that BRAF V600E mutation is associated with lack of response in wild-type KRAS mCRC treated with anti-EGFR MoAbs [1]. More recent analysis have failed to demonstrated a negative predictive response to EGFR inhibitors in BRAF mutated colorectal cancer; however, BRAF is a well described poor prognostic factor.[2]. Overall, the hazard ratios of patients treated with EGFR-blocking antibodies (cetuximab or panitumumab) were not dependent on the BRAF mutation status for overall survival (interaction test P-value: 0.43) but were close to significance for progression-free survival (interaction test P-value: 0.07).[3] The authors concluded that the BRAF mutation was not predictive of benefits provided by anti-EGFR therapies. Similarly, another meta-analysis [4] reported that EGFR-blocking antibodies did not increase the efficacy of standard chemotherapy in BRAF-mutant patients.[4] 1. Mao C, Liao RY, Qiu LX, et al. BRAF V600E mutation and resistance to anti-EGFR monoclonal antibodies in patients with metastatic colorectal cancer: a meta-analysis. Mol Biol Rep. Apr 2011;38(4):2219-2223. PMID 20857202 2. Barras D. BRAF Mutation in Colorectal Cancer: An Update. Biomark Cancer. 2015;7(Suppl 1):9-12. PMID 26396549 3. Rowland A, Dias MM, Wiese MD, et al. Meta-analysis of BRAF mutation as a predictive biomarker of benefit from anti-EGFR monoclonal antibody therapy for RAS wild-type metastatic colorectal cancer. Br J Cancer. Jun 09 2015;112(12):1888-1894. PMID 25989278 4. Pietrantonio F, Petrelli F, Coinu A, et al. Predictive role of BRAF mutations in patients with advanced colorectal cancer receiving cetuximab and panitumumab: a meta-analysis. Eur J Cancer. Mar 2015;51(5):587-594. PMID 25673558
5	Patients with metastatic colorectal carcinoma who have been shown on testing to have variants of BRAF V600E mutations have been found to have poor overall response to anti-EGFR therapy as compared to patients with wild-type. It is critical that only patients with BRAF V600E wild type receive anti-EGFR therapy. An example of this is in one study by Mao et al, the ORR was 29.2% for patients with mutant BRAF compared to 33.5% on wild-type BRAF. BRAF mutational status is a strong predictor for overall survival not only in the metastatic setting but also in earlier stage diagnosis.Studies using the FDA-approved and newer developed LDT tests have found adequate evidence that KRAS mutation analysis reliably and accurately

No.	Response
	detects common BRAF mutations. Results from RT-PCR testing are comparable to next gen sequencing. Testing using immunohistochemical stain (clone VE1) for BRAF V600Ein colon carcinoma needs more data. Some studies have reported near to complete concordance, but there is a report that it is not a useful surrogate for genotyping in colorectal carcinoma. • Mao C, Liao RY, Qiu LX, et al. BRAF V600E mutation and resistance to anti-EGFR monoclonal antibodies in patients with metastatic colorectal cancer: a meta-analysis. Mol Biol Rep. Apr 2011;38(4):2219-2223. PMID 20857202 • Di Nicolantonio F, Martini M, Molinari F, et al. Wild-type BRAF is required for response to panitumumab or cetuximab in metastatic colorectal cancer. J Clin Oncol. Dec 10 2008;26(35):5705-5712. PMID 19001320 • Evaluation of Genomic Applications in Practice Prevention Working Group. Recommendations from the EGAPP Working Group: can testing of tumor tissue for mutations in EGFR pathway downstream effector genes in patients with metastatic colorectal cancer improve health outcomes by guiding decisions regarding anti-EGFR therapy? Genet Med. Jul 2013;15(7):517-527. PMID 23429431 • Jones JC, Grothey A. Understanding BRAF-Mutant Colorectal Cancer. ASCO Daily News 2016 May 26; https://am.asco.org/daily-news/understanding-braf-mutant-colorectal-cancer. Accessed October 26, 2017. • Adackapara CA, Sholl LM, Barletta JA, et al. Immunohistochemistry using the BRAF V600E mutation-specific monoclonal antibody VE1 is not a useful surrogate for genotyping in colorectal adenocarcinoma. Histopathology. Aug 2013;63(2):187-193. PMID 23763264 • Lasota J, Kowalik A, Wasag B, et al. Detection of the BRAF V600E mutation in colon carcinoma: critical evaluation of the immunohistochemical approach. Am J Surg Pathol. Sep 2014;38(9):1235-1241. PMID 24832158
6	I routinely request KRAS, NRAS and BRAF testing in all my stage IV colorectal cancer patients, and consider anti-EGFR therapy in wild-type KRAS and NRAS tumors. At this point, I am not basing my decision to use anti-EGFR agent based on BRAF mutation (although I recognize it portends a poorer prognosis) since I reserve anti-EGFR therapy for 2nd line therapy.
7	The role for BRAF V600E testing as a predictive marker for anti-EGFR monoclonal antibody therapy effectiveness in the treatment of metastatic colorectal cancer is not yet clearly defined. The evidence available does lean to suggest that such antibody therapies are unlikely to be effective in patients whose tumors harbor such a mutation. The meta-analysis from Pietrantonio and colleagues did conclude that BRAF mutation should be considered as a factor against the use of an anti-EGFR monoclonal antibody therapy. Separately, however, the meta-analysis performed by Rowland and colleagues found the evidence for selection for or against an anti-EGFR monoclonal antibody based upon BRAF mutation insufficient. The updated recommendation from the ASCO in 2017 similarly states that the evidence for BRAF testing in this indication is insufficient. There is sparse prospective data to address this issue, and this will be necessary in order to determine if BRAF testing is requisite to the selection of anti-EGFR monoclonal antibody use in metastatic colorectal cancer. We cannot cite personal clinical experiences in a meaningful way, as the instances when we have known the BRAF status of a patient's tumor in this context is quite limited, given that the testing is not routinely assessed. Thus, at present BRAF testing should not be routinely assessed as a biomarker for anti-EGFR selection. Future studies on par with the data establishing RAS testing as such a biomarker (CRYSTAL, OPUS, etc.), could change this, and a similar level of evidence and demonstrated benefit as established the role for RAS testing would be necessary to impart this distinction onto BRAF.Concerning sequences of testing, the value of identifying mutant KRAS in exon 2 in order to predict for or against the use of an anti-EGFR monoclonal antibody for the treatment of metastatic colorectal cancer pre-dates the similar knowledge for the value of mutational status of KRAS exons 3 and 4, NRAS, and, theoretically, BRAF.

No.	Response
	Additionally, of these mutations, KRAS exon 2 mutations are by far the most common. Prior to understanding the relevance of extended RAS testing, many institutions had developed internal tests for the KRAS exon 2 mutations. Rather than develop additional internal testing for the rest of the extended panel, many institutions still assess KRAS exon 2 internally, as it is the most common. If this turns out to be wildtype, internal practice is then to refer the specimen out for commercial testing of the remainder of the panel. Given the likelihood of the mutation being within KRAS exon 2, this practice seems reasonable. Should BRAF ultimately be added to the panel of routinely testing mutations for anti-EGFR monoclonal eligibility, or otherwise be assessed, assessing KRAS exon 2 in a similar fashion is appropriate. • Pietrantonio F, Petrelli F, Coinu A, et al. Predictive role of BRAF mutations in patients with advanced colorectal cancer receiving cetuximab and panitumumab: a meta-analysis. Eur J Cancer. Mar 2015;51(5):587-594. PMID 25673558 • Rowland A, Dias MM, Wiese MD, et al. Meta-analysis of BRAF mutation as a predictive biomarker of benefit from anti-EGFR monoclonal antibody therapy for RAS wild-type metastatic colorectal cancer. Br J Cancer. Jun 09 2015;112(12):1888-1894. PMID 25989278
8	The data concerning the prognostic value of BRAF testing is very clear in that patients whose tumor harbors a BRAF mutation have a much poorer outcome compared to those with wild type BRAF. The predictive value of BRAF testing relative to anti-EGFR therapy is less clear primarily due to the small sample sizes of most clinical trials where this question has been addressed. A recent meta-analysis (Rowland A, Dias MM, Wiese MD, et al. Meta-analysis of BRAF mutation as a predictive biomarker of benefit from anti-EGFR monoclonal antibody therapy for RAS wild-type metastatic colorectal cancer. Br J Cancer. Jun 09 2015;112(12):1888-1894. PMID 25989278) concluded that the data concerning BRAF mutational status in patients with metastatic CRC was insufficient to conclude that benefit from anti-EGFR therapy varied by mutational status of BRAF. However, despite the lack of statistical significance, the data supports a substantial reduction in benefit associated with the use of anti-EGFR therapy in patients with BRAF mutant CRC. Poor prognosis coupled with the reduced benefit associated with the use of anti-EGFR therapy makes knowledge of the BRAF status in patients with metastatic CRC of paramount importance. Given the toxicities and expense associated with the use of anti-EGFR therapy, having knowledge of the BRAF mutational status would help with the clinical decision to use anti-EGFR therapy. In addition, given the relative lack of benefit associated with the use of standard CRC regimens, emerging data support the benefit of either triple therapy (FOLFOXIRI; Cremolini C, Loupakis F, Antoniotti C, et al. FOLFOXIRI plus bevacizumab versus FOLFIRI plus bevacizumab as first-line treatment of patients with metastatic colorectal cancer: updated overall survival and molecular subgroup analyses of the open-label, phase 3 TRIBE study. Lancet Oncol. Oct 2015;16(13):1306-1315. PMID 26338525) or the combination of anti-EGFR plus irinotecan plus a BRAF inhibitor for patients with BRAF mutant CRC (Kopetz S, McDonough SL, Lenz H-J, et al. Randomized trial of irinotecan and cetuximab with or without vemurafenib in BRAF-mutant metastatic colorectal cancer (SWOG S1406) [abstract]. J Clin Oncol. 2017;35(15 Suppl):3505). Taken together, these data support the value of BRAF mutational analysis in clinical decision making.
9	There is evidence that patients with metastatic colorectal cancer with BRAF V600E variants do not benefit from treatment with EGFR inhibitors. While BRAF V600E is a known prognostic factor, we also know that response rates to almost any of our standard therapies are low, and this includes EGFR inhibitors. Frontline, phase III, randomized metastatic CRC studies showing this are listed below: • Final results from PRIME: randomized phase III study of panitumumab with FOLFOX4 for first-line treatment of metastatic colorectal cancer. Douillard JY, Siena S, Cassidy J, et al. Final results from PRIME: randomized phase III study of panitumumab with FOLFOX4 for

No.	Response
	first-line treatment of metastatic colorectal cancer. Ann Oncol. Jul 2014;25(7):1346-1355. PMID 24718886 • Van Cutsem E, Kohne CH, Hitre E, et al. Cetuximab and chemotherapy as initial treatment for metastatic colorectal cancer. N Engl J Med. Apr 02 2009;360(14):1408-1417. PMID 19339720 In addition, a recent published abstract (Kopetz S, McDonough SL, Lenz H-J, et al. Randomized trial of irinotecan and cetuximab with or without vemurafenib in BRAF-mutant metastatic colorectal cancer (SWOG S1406) [abstract]. J Clin Oncol. 2017;35(15 Suppl):3505.) comparing cetuximab/irinotecan with cetuximab/vemurafenib/irinotecan showed a PFS of the cetuximab/irinotecan arm of 2 months, suggesting a lack of benefit of standard therapy (chemo + EGFR inhibition).I agree with the NCCN assertion that patients with BRAF mutated tumors are highly unlikely to respond to anti-EGFR therapy.
10	• BRAF mutations associated with low probability response to epidermal growth factor receptor (EGFR) inhibitors. • BRAF V600E associated with worse prognosis. o High microsatellite instability (MSI), could be candidate for immunotherapy. o Non V600E BRAF associated with better prognosis. All these are important for prognosis and treatment of patients with colorectal cancer.

- Based on the evidence and your clinical experience for *BRAF* V600E variant testing to guide treatment with *EGFR*-targeted therapy in individuals with metastatic CRC:
  - Respond YES or NO whether the intervention would be expected to provide a clinically meaningful benefit in the net health outcome.
  - Use the 1 to 5 scale outlined below to indicate your level of confidence that there is adequate evidence that supports your conclusions.

No.	Yes/No	Low Confidence		Intermediate Confidence		High Confidence
		1	2	3	4	5
1	Yes			X		
2	Yes				X	
3	Yes				X	
4	Yes			X		
5	Yes				X	
6	No				X	
7	Yes			X		
8	Yes					X
9	Yes			X		
10	Yes					X

- Based on the evidence and your clinical experience for *BRAF* V600E variant testing to guide treatment with *EGFR*-targeted therapy in individuals with metastatic CRC:

- Respond YES or NO for each indication whether this intervention is consistent with generally accepted medical practice.
- Use the 1 to 5 scale outlined below to indicate your level of confidence in your conclusions.

No.	Yes/No	Low Confidence		Intermediate Confidence		High Confidence
		1	2	3	4	5
1	Yes			X		
2	Yes			X		
3	Yes					X
4	Yes			X		
5	Yes				X	
6	No				X	
7	No				X	
8	Yes				X	
9	Yes			X		
10	Yes			X		

- Additional comments and/or any citations supporting your clinical input on the use of *BRAF* V600E variant testing to guide treatment with EGFR-targeted therapy in individuals with metastatic CRC.

No.	Additional Comments
1	The utilization and importance of BRAF V600 variant testing in patients with metastatic colon cancer extends beyond guiding treatment with EGFR-targeted therapy. Thus we recommend that Evidence Street expand the meaningful clinical benefit for BRAF in the evidence summary beyond selecting a specific targeted treatment. AMP has high confidence that BRAF V600 variant testing is clinically beneficial for these patients. BRAF V600 variant testing should not be denied for these patients solely on the basis of EGFR treatment selection. We disagree with the evidence summary that evidence is insufficient to determine the effects of BRAF variant testing on health outcomes. As mentioned above, the ASCP/CAP/AMP/ASCO guideline conducted four systematic reviews and three systematic reviews that included meta-analyses pertaining to the prognostic and predictive value of BRAF mutations in patients with CRC were identified through a systematic review process. See Table 8 and Supplemental Table 14 in the guidelines. These studies revealed that patients with advanced CRC who possess a BRAF mutation have significantly poorer outcomes as measured by PFS and OS and have a decreased response rate to anti-EGFR therapy relative to those with nonmutated BRAF. Thus, knowledge of a patient's BRAF mutation status is important since these patients have particularly poor prognosis and any therapies should be correspondingly aggressive. Further, molecular testing for BRAF variants is also supported by NCCN guidelines. The evidence summary states on page 17 that direct evidence is limited for BRAF variant

No.	Additional Comments
	testing due to the low prevalence of BRAF mutations in CRC. This is not the case, in fact BRAF activating mutations occur in about 8% of advanced disease patients with CRC and in approximately 14% of patients with localized stage II and III CRC. As such, mutations in BRAF constitute a substantial subset of patients with CRC. Evidence to support his statement: • Gavin PG, Colangelo LH, Fumagalli D, et al. Mutation profiling and microsatellite instability in stage II and III colon cancer: an assessment of their prognostic and oxaliplatin predictive value. Clin Cancer Res. Dec 01 2012;18(23):6531-6541. PMID 23045248 • Xu Q, Xu AT, Zhu MM, et al. Predictive and prognostic roles of BRAF mutation in patients with metastatic colorectal cancer treated with anti-epidermal growth factor receptor monoclonal antibodies: a meta-analysis. J Dig Dis. Aug 2013;14(8):409-416. PMID 23615046 • Yuan ZX, Wang XY, Qin QY, et al. The prognostic role of BRAF mutation in metastatic colorectal cancer receiving anti-EGFR monoclonal antibodies: a meta-analysis. PLoS One. 2013;8(6):e65995. PMID 23776587 • Forbes SA, Bhamra G, Bamford S, et al. The Catalogue of Somatic Mutations in Cancer (COSMIC). Curr Protoc Hum Genet. Apr 2008;Chapter 10:Unit 10 11. PMID 18428421
2	None Listed
3	Treating metastatic colorectal cancer is becoming increasingly individualized. Individuals with BRAF V600E mutations represent 5 to 10% of patients in various series. There is yet a critical mass of data to be definitive, but in my practice, we do not utilize anti-EGFR therapy in this subgroup of patients. Current data indicates BRAF V600E variants having a more aggressive course with lack of response to anti-EGFR therapy. Nevertheless, data does exist as presented by Kopetz et al at ASCO that combining anti-EGFR therapy (Cetuximab with MEK inhibitor (vemurafenib) and irinotecan improved progression-free survival. Similar trials with other agents are also underway. The analogy here may well be similar to Her 2 testing in the past with its associated poor prognosis until development of anti Her-2 therapy. I believe that there is reasonably good data now on the value proposition of including BRAF mutation analysis on all metastatic specimens RAS wild. Should a mutation be found for BRAF in a RAS wild patient, alternative treatment options need to be considered. • Kopetz S, McDonough SL, Morris VK, et al. Randomized trial of irinotecan and cetuximab with or without vemurafenib in BRAF-mutant metastatic colorectal cancer (SWOG 1406) [abstract]. J Clin Oncol. 2017;35(4 Suppl):520. • Tabernero J, Van Geel R, Guren TK, et al. Phase 2 results: Encorafenib (ENCO) and cetuximab (CETUX) with or without alpelisibs (ALP) in patients with advanced BRAF-mutant colorectal cancer (BRAFm CRC) [abstract]. J Clin Oncol. 2016;34(15 Suppl):3544
4	See above response in Question 1.
5	Testing using next gen sequencing has found several non-V600E mutations. Additional studies need to be done on these non-V600E mutations to determine its significance and effect on patient's response to therapy.
6	I don't use BRAF test to determine use of anti-EGFR therapy.
7	An important issue to consider for future use of BRAF testing is cost. One cycle of cetuximab at our institution would cost over $11,000 to administer, which in most instances would already surpass the cost of the BRAF mutational status testing. Thus, if the value of BRAF mutational testing as a predictor for or against anti-EGFR monoclonal antibody therapy is confirmed, cost/benefit would also be a key reason to quickly adopt its use.

No.	Additional Comments
8	• Sepulveda AR, Hamilton SR, Allegra CJ, et al. Molecular Biomarkers for the Evaluation of Colorectal Cancer: Guideline From the American Society for Clinical Pathology, College of American Pathologists, Association for Molecular Pathology, and the American Society of Clinical Oncology. J Clin Oncol. May 01 2017;35(13):1453-1486. PMID 28165299
9	None Listed
10	None Listed

- Is there any evidence missing from the attached draft review of evidence that demonstrates clinical benefit?

No	Yes/No	Citations of Missing Evidence
1	Yes	Yes, in March 2017, the American Society for Clinical Pathology, College of American Pathologists, Association for Molecular Pathology, and American Society of Clinical Oncology published an updated guideline on Molecular Biomarkers for the Evaluation of Colorectal Cancer. This is an evidence-based guideline recommendation, which was constructed through a systematic review of the literature to establish standard molecular biomarker testing of CRC tissue to guide EGFR therapies and conventional chemotherapy regimens. An expert panel was convened to develop an evidence-based guideline to establish standard molecular biomarker testing and guide therapies for patients with CRC. During this process, a comprehensive literature search that included more than 4,000 articles was conducted. The guideline is available for download here and is open access. A full citation is also provided below, for your convenience. http://dx.doi.org/10.1016/j.jmoldx.2016.11.001 • Sepulveda AR, Hamilton SR, Allegra CJ, et al. Molecular Biomarkers for the Evaluation of Colorectal Cancer: Guideline From the American Society for Clinical Pathology, College of American Pathologists, Association for Molecular Pathology, and American Society of Clinical Oncology. J Mol Diagn. Mar 2017;19(2):187-225. PMID 28185757 The evidence summary states that no published studies are available demonstrating the analytic validity of LDTs for KRAS variants, but only for the FDA approved therascreen KRAS RGQ PCR Kit and Cobas KRAS Mutation Test. Evidence that KRAS mutation status was predictive of response to anti EGFR therapies first emerged around 2008. Those studies utilized LDTs as have virtually all subsequent clinical studies. FDA approved assays specific for KRAS codons 12 and 13 did not become available until 2012. In the interim, KRAS testing was performed by LDTs as regulated under CLIA without evidence of inadequacy. An FDA approved assay for expanded RAS testing did not become available until June 2017. Further, it's important to note that the clinical studies that established expanded RAS testing clinically did not use FDA approved assays. Thus, it is inaccurate to state in the summary that there is a lack of published evidence on the analytic validity to detect RAS variants. Below are a few examples of published evidence: • Whitehall V, Tran K, Umapathy A, et al. A multicenter blinded study to evaluate KRAS mutation testing methodologies in the clinical setting. J Mol Diagn. Nov 2009;11(6):543-552. PMID 19815694 • Weichert W, Schewe C, Lehmann A, et al. KRAS genotyping of paraffin-embedded colorectal cancer tissue in routine diagnostics: comparison of methods and impact of histology. J Mol Diagn. Jan 2010;12(1):35-42. PMID 20007841

No	Yes/No	Citations of Missing Evidence
		• Kamel-Reid S, Zhang T, Persons DL, et al. Validation of KRAS testing for anti-EGFR therapeutic decisions for patients with metastatic colorectal carcinoma. Arch Pathol Lab Med. Jan 2012;136(1):26-32. PMID 22208484 • Kaul KL, Sabatini LM, Tsongalis GJ, et al. The Case for Laboratory Developed Procedures: Quality and Positive Impact on Patient Care. Acad Pathol. Jan-Dec 2017;4:2374289517708309. PMID 28815200 Further, the evidence summary lists two FDA-approved tests for KRAS variant analysis, the Cobas KRAS mutation test and the therascreen KRAS RGQ PCR kits. In June 2017, FDA granted market approval to the Praxis Extended RAS Panel and should be included as an approved companion diagnostic tests for KRAS and NRAS variant analysis. It should also be noted in the evidence summary that the cobas KRAS mutation test and the therascreen KRAS RGQ PCR kits do not detect all the variants for KRAS and NRAS recommended by current guidelines.https://www.fda.gov/drugs/informationondrugs/approveddrugs/ucm565785.htm
2	No	
3	No	
4	Yes	Results of the phase II Southwest Oncology Group (SWOG) 1406 trial presented at the ASCO Gastrointestinal Cancer Symposium in January 2017 reported that in patients with metastatic colorectal cancer who have mutations in BRAF V600, the addition of the BRAF inhibitor vemurafenib (Zelboraf) to cetuximab (Erbitux) and irinotecan significantly improved progression-free survival. The trial met its primary endpoint, improving median progression-free survival from 2.0 months with cetuximab/irinotecan to 4.4 months with the addition of vemurafenib (HR = 0.42, P = 0.0002). Grade 3/4 adverse events were significantly higher in the experimental arm and included neutropenia (28% vs 7%), anemia (13% vs 0%), and nausea (15% vs 0%). Arthralgias (a known side effect of vemurafenib) were numerically increased. There was no increase in skin toxicity or fatigue with the addition of vemurafenib. Treatment discontinuation due to adverse events occurred in 18% of the experimental arm and 8% of the control arm. Almost 50% of patients in the control arm crossed over at the time of disease progression. Overall survival and efficacy at crossover data remain immature.[1] Moreover, results from the Phase 3 BEACON CRC study evaluating binimetinib, a MEK inhibitor, encorafenib, a BRAF inhibitor and Erbitux® (cetuximab), an anti-EGFR antibody, in patients with BRAF-mutant colorectal cancer (CRC) whose disease has progressed after one or two prior regimens in the metastatic setting were presented at ESMO 2017 in September. There was a 41% confirmed ORR for patients on combination of binimetinib, encorafenib and cetuximab. In the safety lead-in, the triplet combination was generally well-tolerated. The most common grade 3 or 4 adverse events (AEs) seen in at least 10% of patients were nausea (10%), vomiting (10%), increased blood creatine kinase (10%) and urinary tract infection (10%). Three patients discontinued treatment due to AEs with only one considered related to treatment. At the time of the analysis, 76% of patients remain on study treatment after a median duration of treatment of 5.6 months (range 1.0 - 9.3 months). [2] 1. Kopetz S, McDonough SL, Morris VK, et al. Randomized trial of irinotecan and cetuximab with or without vemurafenib in BRAF-mutant metastatic colorectal cancer (SWOG 1406) [abstract]. J Clin Oncol. 2017;35(4 Suppl):520.

No	Yes/No	Citations of Missing Evidence
		2. Huijberts S, Schellens JHM, Fakih M, et al. BEACON CRC (binimetinib [BINI], encorafenib [ENCO], and cetuximab [CTX] combined to treat BRAF-mutant metastatic colorectal cancer [mCRC]): A multicenter, randomized, open-label, three-arm phase III study of ENCO plus CTX plus or minus BINI vs irinotecan (IRI)/CTX or infusional 5-fluorouracil/folinic acid/IRI (FOLFIRI)/CTX with a safety lead-in of ENCO + BINI + CTX in patients (Pts) with BRAFV600E mCRC [abstract]. J Clin Oncol. 2017;35(15 Suppl).
5	No	
6	No	
7	Yes	There may be benefit to patients via RAF inhibition. Data has been presented evaluating the combination of irinotecan and cetuximab with or without the RAF-inhibitor vemurafenib in the treatment of patients with BRAF-mutant colorectal cancer. This phase II clinical trial enrolled 106 patients with metastatic colorectal cancer whose tumors harbored a BRAF V600E mutation. Patients were randomized to receive either cetuximab + irinotecan or cetuximab + irinotecan + vemurafenib.PFS was improved with the addition of vemurafenib in this population (4.4 months vs 2.0 months) as was disease control rate (67% vs 22%). The conclusions of this study suggest that adding a BRAF inhibitor to irinotecan + cetuximab (resulting in simultaneous BRAF and EGFR inhibition) is effective in these patients. This option for treatment is being actively investigated and, if validated, would certainly change the value of BRAF testing on a routine basis for these patients. • Kopetz S, McDonough SL, Morris VK, et al. Randomized trial of irinotecan and cetuximab with or without vemurafenib in BRAF-mutant metastatic colorectal cancer (SWOG 1406) [abstract]. J Clin Oncol. 2017;35(4 Suppl):520.
8	Yes	As noted above in responses to #1 and 4
9	No	
10	NR	

NR: no response.