

Medical Policy



Title: KRAS, NRAS, and BRAF Variant Analysis in Metastatic Colorectal Cancer

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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

Populations	Interventions	Comparators	Outcomes
Individuals: <ul style="list-style-type: none"> • With metastatic colorectal cancer 	Interventions of interest are: <ul style="list-style-type: none"> • <i>BRAF</i> variant testing to guide treatment 	Comparators of interest are: <ul style="list-style-type: none"> • No <i>BRAF</i> variant testing to guide treatment 	Relevant outcomes include: <ul style="list-style-type: none"> • Overall survival • Disease-specific survival • Change in disease status • Medication use • Resource utilization • Treatment-related morbidity

DESCRIPTION

The epidermal growth factor receptor (EGFR) is overexpressed in colorectal cancer (CRC). EGFR-targeted therapy, with the monoclonal antibodies cetuximab and panitumumab, has shown 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.

OBJECTIVE

The objective of this policy is to determine whether genetic testing for *KRAS*, *NRAS*, and *BRAF* improves the net health outcome in individuals with metastatic colorectal cancer by predicting treatment response.

BACKGROUND

Cetuximab (Erbix, ImClone Systems) and panitumumab (Vectibix, Amgen) are monoclonal antibodies that bind to the 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. RAS proteins are G proteins that cycle between active (RAS-GTP) and inactive (RAS-GDP) forms, in response to stimulation from a cell surface receptor such as EGFR, and act as a binary switch between the cell surface EGFR and downstream signaling pathways. The *KRAS* gene can harbor oncogenic mutations that result in a constitutively activated protein, independent of EGFR ligand binding, rendering antibodies to the upstream EGFR ineffective. Approximately 40% of colorectal cancers 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 relatively rare compared with *KRAS*, detected in perhaps 2% to 7% of CRC specimens. It is unclear whether *NRAS* mutations predict poor response to anti-EGFR monoclonal antibody therapy or are prognostic of poor CRC outcome in general. A third proto-oncogene, *BRAF*, encodes a protein kinase and is involved in intracellular signaling and cell growth and is a principal downstream effector of *KRAS*. *BRAF* mutations occur in less than 10% to 15% of CRCs and appear to

be a marker of poor prognosis. *KRAS* and *BRAF* mutations are considered to be mutually exclusive.

Cetuximab and panitumumab have FDA marketing approval for treatment of metastatic CRC in the refractory disease setting. FDA approval for panitumumab indicates that panitumumab is not indicated for the treatment of patients with *KRAS* or *NRAS* mutation-positive disease in combination with oxaliplatin-based chemotherapy.¹

REGULATORY STATUS

Approved Companion Diagnostic Tests for *KRAS* Variant Analysis

Companion diagnostic tests for the selection of cetuximab and panitumumab have been approved by FDA through the premarket approval process, including:

"The cobas® *KRAS* Mutation Test, for use with the cobas® 4800 System, [which] is a real-time PCR [polymerase chain reaction] test for the detection of seven somatic mutations in codons 12 and 13 of the *KRAS* gene in DNA derived from formalin-fixed paraffin-embedded human colorectal cancer (CRC) tumor tissue. The test is intended to be used as an aid in the identification of CRC patients for whom treatment with Erbitux® (cetuximab) or with Vectibix® (panitumumab) may be indicated based on a no mutation detected result."²

"The theascreen® *KRAS* RGQ PCR Kit is a real-time qualitative PCR assay used on the Rotor-Gene Q MDx instrument for the detection of seven somatic mutations in the human *KRAS* oncogene, using DNA extracted from formalin-fixed paraffin-embedded (FFPE), colorectal cancer (CRC) tissue. The theascreen *KRAS* RGQ PCR Kit is intended to aid in the identification of CRC patients for treatment with Erbitux (cetuximab) and Vectibix (panitumumab) based on a *KRAS* no mutation detected test result."²

Laboratory-Developed Tests for *KRAS*, *NRAS*, and *BRAF* Variant Analysis

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. *KRAS*, *NRAS*, and *BRAF* variant analyses using polymerase chain reaction methodology are available under the auspices of the Clinical Laboratory Improvement Amendments. Laboratories that offer laboratory-developed tests must be licensed under the Clinical Laboratory Improvement Amendments for high-complexity testing. To date, 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.

Policy Guidelines

There is support from the evidence and clinical input to use *BRAF V600* variant testing for prognostic stratification. Clinical input suggests that patients who are positive for this variant may be considered for clinical trials.

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.

Genetics Nomenclature Update

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

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

Table PG1. Nomenclature to Report on Variants Found in DNA

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

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

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

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

RATIONALE

The most recent literature review was performed for the period through May 10, 2018.

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.

A large body of literature has shown that metastatic colorectal cancer (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, in exons 3 (codons 59 and 61) and exon 4 (codons 117 and 146), and variants in *NRAS* exon 2 (codons 12 and 13), exons 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.

***KRAS* Variant Testing to Guide Treatment for Metastatic CRC**

Clinical Context and Test Purpose

The purpose of *KRAS* variant testing in individuals with metastatic 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 PICOTS were used to select literature to inform this review.

Patients

The relevant population of interest includes 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) and overall survival (OS).

Timing

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

Setting

Patients with metastatic CRC are actively managed by oncologists.

Technically Reliable

Assessment of technical reliability focuses on specific tests and operators and requires review of unpublished and often proprietary information. Review of specific tests, operators, and unpublished data are outside the scope of this evidence review and alternative sources exist. This evidence review focuses on the clinical validity and clinical utility.

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).

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

RCTs have performed nonconcurrent subgroup analyses of the efficacy of EGFR inhibitors in patients with wild-type vs 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.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 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 of 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. PFS and tumor response rate were assessed radiographically every 4 to 8 weeks until disease progression using Response Evaluation Criteria in Solid Tumors criteria by blinded, central review. In the *KRAS*-assessable population, 20% of patients had a treatment-related grade 3 or 4 adverse events. As shown in Table 1, 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 1. *KRAS* Status and Efficacy of Panitumumab as Monotherapy in the Treatment of Chemotherapy-Refractory Metastatic Colorectal Cancer (N=427)

Outcomes	<i>KRAS</i> WT (n=243 [57%])		<i>KRAS</i> MT (n=184 [43%])	
	P (n=124)	BSC (n=119)	P (n=84)	BSC (n=100)
Median progression-free survival, wk	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

BSC: best supportive care; CI: confidence interval; MT: mutated; P: panitumumab; 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 end points 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 vs 7.9 weeks for mutated; hazard ratio [HR], 0.32; 95% confidence interval [CI], 0.22 to 0.45).

After completion of the CRYSTAL trial (detailed below), in which 1198 patients with metastatic CRC were randomized to cetuximab in combination with folinic acid (leucovorin), 5-fluorouracil, 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 PCR 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. PFS and tumor response rate 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 2.

Table 2. *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		<i>KRAS</i> WT (n=348 [64%] ^b)		<i>KRAS</i> 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, mo ^c	8.9	8.0	9.9	8.7	7.6	8.1
Hazard ratio	0.68 (p=0.017)				1.07 (p=0.47)	

Adapted from Van Cutsem et al (2009).⁶

C: cetuximab; CI: confidence interval; F: FOLFIRI (folinic acid, 5-fluorouracil, and irinotecan); HR: hazard ratio; 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 OPUS trial, the intention-to-treat (ITT) population consisted of 337 patients randomized to cetuximab and folinic acid (leucovorin), 5-fluorouracil, 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 or baseline characteristics 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 3 summarizes study findings.

Table 3. *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	<i>KRAS</i> WT (n=134 [58%])		<i>KRAS</i> MT (n=99 [42%])	
	C+Fx	Fx	C+Fx	Fx
n (<i>KRAS</i> -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)

Outcomes	<i>KRAS</i> WT (n=134 [58%])		<i>KRAS</i> MT (n=99 [42%])	
p	0.011		0.106	
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	0.016		0.019	
Hazard ratio	0.57		1.83	

Adapted from Bokemeyer et al (2009).⁷

C: cetuximab; CI: confidence interval; Fx: FOLFOX (folinic acid, 5-fluorouracil, 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 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=0.04). 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=0.003; OS: 17.2 months vs 24.9 months, respectively, p=0.03). 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 had 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, and 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<0.001) 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<0.001). 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=0.89) or PFS (HR=0.99, p=0.96).

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 or to FOLFOX4.¹⁰ The primary end point was PFS; OS was a secondary end point. 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=0.02). A nonsignificant increase in OS was also observed for panitumumab plus FOLFOX4 vs FOLFOX4 (median OS, 23.9 months vs 19.7 months, respectively; HR=0.83; 95% CI, 0.67 to 1.02; p=0.072). 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=0.02), and median OS was 15.5 months vs 19.3 months, respectively (HR=1.24; 95% CI, 0.98 to 1.57; p=0.068). 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) demonstrated that the addition of cetuximab to FOLFIRI statistically 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=0.009), PFS (median, 9.9 months vs 8.4 months; HR=0.696; p=0.001), and response rate (57.3% vs 39.7%; odds ratio [OR], 2.069; p<0.001) compared with FOLFIRI alone. Significant interactions between *KRAS* status and treatment effect were noted for all key efficacy end points. *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 end points 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 FOLFIRI alone as a second-line treatment.¹² The trial end points 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=0.004); 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=0.12). 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 (MRC COIN 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%) of 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=0.67). *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=0.006), PFS (HR=0.66; p<0.001), and ORR (OR=2.16; p<0.001). *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 end points 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 3 randomized trials discussed, a number of single-arm studies have retrospectively evaluated *KRAS* variant status and treatment response in patients with metastatic CRC.¹⁷⁻²¹ 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}

Section Summary: Clinically Valid

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.

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: Clinically Useful

Direct evidence for the clinical validity of *KRAS* variant testing includes RCTs. RCTs supporting 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 CRC**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 PICOTS were used to select literature to inform this review.

Patients

The relevant population of interest includes 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 and OS.

Timing

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

Setting

Patients with metastatic CRC are actively managed by oncologists.

Technically Reliable

Assessment of technical reliability focuses on specific tests and operators and requires review of unpublished and often proprietary information. Review of specific tests, operators, and unpublished data are outside the scope of this evidence review and alternative sources exist. This evidence review focuses on the clinical validity and clinical utility.

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.

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.²³⁻²⁵ Reviewers suggested that the pooled analyses showed a trend toward a poor OR 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

RCTs 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 end points 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 4 summarizes the PFS and OS HRs for panitumumab plus FOLFIRI vs FOLFIRI alone. The HRs more strongly favored panitumumab in the wild-type *RAS* population.

Table 4. Hazard Ratios of Panitumumab Plus FOLFIRI vs FOLFIRI Alone Based on *RAS* Status

<i>RAS</i> Status	PFS HR (95% CI)	p	OS HR (95% CI)	p
Wild-type <i>RAS</i>	0.70 (0.54 to 0.91)	0.007	0.81 (0.63 to 1.03)	0.08
Wild-type <i>KRAS</i> exon 2	0.73 (0.59 to 0.90)	0.004	0.85 (0.70 to 1.04)	0.12

CI: confidence interval; FOLFIRI: (folinic acid, 5-fluorouracil, 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 vs 10% when treated with FOLFIRI alone. Therefore, *RAS* wild-type status predicted 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; $p=0.47$) and median OS (16.4 months vs 17.7 months; $p=0.64$). Patients with tumors without *RAS* variants experienced significant benefit in median PFS (9.9 months vs 8.4 months; $p<0.05$) and median OS (23.5 months vs 20 months; $p<0.05$) 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; $p=0.03$). 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; $p=0.01$). 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; $p<0.001$) 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=0.379). A treatment interaction analysis was suggestive but not significantly indicative of an interaction between the presence of mutated *NRAS* and poorer outcome (p=0.076). 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=0.013). However, there were no significant differences between *NRAS* mutant and wild-type genes in median PFS (14 weeks vs 26 weeks, p=0.055) or OS (38 weeks vs 50 weeks, p=0.051). 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 PCR 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.

Section Summary: Clinically Valid

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.

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: Clinically Useful

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 CRC**

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 PICOTS were used to select literature to inform this review.

Patients

The relevant population of interest includes 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 and OS.

Timing

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

Setting

Patients with metastatic CRC are actively managed by oncologists.

Technically Reliable

Assessment of technical reliability focuses on specific tests and operators and requires review of unpublished and often proprietary information. Review of specific tests, operators, and unpublished data are outside the scope of this evidence review and alternative sources exist. This evidence review focuses on the clinical validity and clinical utility.

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).

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=0.33) or ORR (RR=1.31; 95% CI, 0.83 to 2.08; p=0.25) 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 end point 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.³¹⁻⁴⁰ All were conducted retrospectively (1 study was a nonconcurrent analysis of response in a population previously randomized⁴⁰). 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=0.048). 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, 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's meta-analysis (2011) 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 end point 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, and none of whom responded to chemotherapy, compared with 32% (24/74) of patients with wild-type *BRAF* who did. In the CAIRO2 study, Tol et al (2009) retrospectively 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 ($p=0.744$).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; $p=0.001$). PFS for *BRAF*-mutated vs wild-type patients was a median of 8 weeks vs 26 weeks, respectively (HR=3.74; 95% CI, 2.44 to 5.75; $p<0.001$), and median OS was 26 weeks vs 54 weeks, respectively (HR=3.03; 95% CI, 1.98 to 4.63; $p<0.001$).

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 that *BRAF* tumor variant was a strong indicator of poor prognosis for all efficacy end points compared with those whose tumors were wild-type.

Section Summary: Clinically Valid

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.

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: Clinically Useful

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.

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 overall survival, 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 RCTs 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 and supports 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 a meaningful 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 RCTs and retrospective cohort studies. Relevant outcomes are overall survival, 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 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 a meaningful 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 RCTs. Relevant outcomes are overall survival, 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 the effects of the technology on health outcomes.

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

In response to requests, clinical input on [indications] was received from 11 respondents, including 2 specialty society-level response, 1 physician from the academic center, and 6 physicians from 2 health systems, while this policy was under review in 2017.

Based on the evidence and independent clinical input, the clinical input supports that the following indication provides a clinically meaningful improvement in the net health outcome and is consistent with generally accepted medical practice:

- Use of *BRAF* V600E variant analysis in individuals with metastatic colorectal cancer who are found to be wild-type on *KRAS* and *NRAS* variant analysis to guide management decisions.

PRACTICE GUIDELINES AND POSITION STATEMENTS

National Comprehensive Cancer Network

National Comprehensive Cancer Network guidelines on the treatment of colon cancer recommend that tumor tissue should be genotyped for *RAS* (*KRAS* and *NRAS*) and *BRAF* variants for all patients with metastatic colon cancer (v.2.2018).⁴² Testing should be performed on archived specimens of primary tumor or a metastasis at the time of diagnosis of metastatic disease. The guidelines indicate that cetuximab and panitumumab are appropriate only for patients with a tumor that expresses wild-type *KRAS* and *NRAS* genes. Individuals with *KRAS* variant in exons 2, 3, or 4, or with *NRAS* variant in exons 2, 3, or 4, are not eligible for treatment with cetuximab or panitumumab. The guidelines also state that the presence of the *BRAF* V600E variant makes response to panitumumab and cetuximab highly unlikely. However, the concurrent administration of a *BRAF* inhibitor may make a response to these treatments more likely.

American College of Medical Genetics and Genomics

An evidence review published in 2013 by the American College of Medical Genetics and Genomics, *Evaluation of Genomic Applications in Practice and Prevention (EGAPP) Working Group*, states that evidence is insufficient to support the clinical validity or utility of testing CRC specimens for *NRAS* mutations to guide patient management.⁴³ In the same review, EGAPP found no guidelines on *NRAS* testing from any other U.S. group.

American Society of Clinical Oncology

In 2017, American Society of Clinical Oncology along with American Society for Clinical Pathology, College of American Pathologists, Association for Molecular Pathology published guidelines on Molecular Biomarkers for the Evaluation of Colorectal Cancer.⁴⁴

Table 5. Summary of Recommendations

Guideline Statements	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 a 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

Guideline Statements	Type	SOE	QOE
There is insufficient evidence to recommend BRAF c.1799 p.V600 mutational status as a predictive molecular biomarker for response to anti-EGFR inhibitors	No recommendation	Insufficient, benefits/harms balance unknown	Insufficient

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

The American Society of Clinical Oncology published a provisional clinical opinion update in 2016 on extended *RAS* variant testing in metastatic colorectal cancer to predict response to anti-EGFR monoclonal antibody therapy.⁴⁵ 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 are small, and there is inadequate evidence to provide a definitive opinion on the lack of benefit for the use of anti-EGFR 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 seemed to confer the same lack of benefit with anti-EGFR therapy as seen with the more common variants in exon 2 of *KRAS*.

U.S. PREVENTIVE SERVICES TASK FORCE RECOMMENDATIONS

Not applicable.

ONGOING AND UNPUBLISHED CLINICAL TRIALS

A search of ClinicalTrials.gov in June 2018 did not identify any ongoing or unpublished trials that would likely influence this review.

CODING

The following codes for treatment and procedures applicable to this policy are included below for informational purposes. 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.

CPT/HCPCS

- 81210 BRAF (B-Raf proto-oncogene, serine/threonine kinase) (eg, colon cancer, melanoma), gene analysis, V600E 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)
- 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)

- There are specific CPT codes for *BRAF*, *KRAS*, or *NRAS* variant analysis: 81210, 81275, 81276, 81311.
- There is also a CPT code for using archival tissue for molecular analysis: 88363.

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: <ul style="list-style-type: none"> Added CPT codes: 81276, 81311. Revised nomenclature of codes: 81210, 81275.
08-29-2016	Updated Description section. <p>In Policy section:</p> <ul style="list-style-type: none"> 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 "<i>NRAS</i> 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." <p>Updated Rationale section.</p> <p>In Coding section:</p> <ul style="list-style-type: none"> Removed CPT codes: 81403, 81404. <p>Updated References section.</p>
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." <p>Updated Description section.</p> <p>In Policy section:</p> <ul style="list-style-type: none"> In Item A, removed "mutation" and added "variant" and "epidermal growth factor" to read, "<i>KRAS</i> 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, "<i>NRAS</i> 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, "<i>BRAF</i> variant analysis may be considered medically necessary for patients with metastatic colorectal cancer who are

	found to be wild-type on <i>KRAS</i> and <i>NRAS</i> variant analysis to guide management decisions." <ul style="list-style-type: none"> ▪ Added Policy Guidelines.
	Updated Rationale section.
	In Coding section: <ul style="list-style-type: none"> ▪ Removed ICD-9 codes.
	Updated References section.
	Added Appendix section.
08-29-2018	Updated Description section.
	Updated Rationale section.
	Updated References section.
	Removed Appendix.

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