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

Policy Number: 2.04.53  Last Review: 5/2018

Policy
Blue Cross and Blue Shield of Kansas City (Blue KC) will provide coverage for KRAS variant analysis in metastatic colorectal cancer when it is determined to be medically necessary because the criteria shown below are met.

Note: Genetic testing may be excluded in some contracts. Verify benefits prior to review of Medical Necessity.

When Policy Topic is covered
KRAS mutation 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.

NRAS mutation 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.

BRAF variant analysis 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.

When Policy Topic is not covered
KRAS mutation analysis is considered investigational if the criterion is not met.

Considerations
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**

<table>
<thead>
<tr>
<th>Previous</th>
<th>Updated</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mutation</td>
<td>Disease-associated variant</td>
<td>Disease-associated change in the DNA sequence</td>
</tr>
<tr>
<td>Variant</td>
<td>Change in the DNA sequence</td>
<td></td>
</tr>
</tbody>
</table>
| 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**

<table>
<thead>
<tr>
<th>Variant Classification</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pathogenic</td>
<td>Disease-causing change in the DNA sequence</td>
</tr>
<tr>
<td>Likely pathogenic</td>
<td>Likely disease-causing change in the DNA sequence</td>
</tr>
<tr>
<td>Variant of uncertain significance</td>
<td>Change in DNA sequence with uncertain effects on disease</td>
</tr>
<tr>
<td>Likely benign</td>
<td>Likely benign change in the DNA sequence</td>
</tr>
<tr>
<td>Benign</td>
<td>Benign change in the DNA sequence</td>
</tr>
</tbody>
</table>

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

**Coding**

There are specific CPT codes for *BRAF, KRAS, or NRAS* variant analysis.

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: 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).

There is also a CPT code for using archival tissue for molecular analysis:

88363: Examination and selection of retrieved archival (ie, previously diagnosed) tissue(s) for molecular analysis (eg, *KRAS* mutational analysis).

### Description of Procedure or Service

<table>
<thead>
<tr>
<th>Populations</th>
<th>Interventions</th>
<th>Comparators</th>
<th>Outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Individuals:</td>
<td>Interventions of interest are:</td>
<td>Comparators of interest are:</td>
<td>Relevant outcomes include:</td>
</tr>
<tr>
<td>• With metastatic colorectal cancer</td>
<td><em>KRAS</em> mutation testing to guide treatment</td>
<td>• No <em>KRAS</em> mutation testing to guide treatment</td>
<td>• Overall survival</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Disease-specific survival</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Change in disease status</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Medication use</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Resource utilization</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Treatment-related morbidity</td>
</tr>
<tr>
<td>Individuals:</td>
<td>Interventions of interest are:</td>
<td>Comparators of interest are:</td>
<td>Relevant outcomes include:</td>
</tr>
<tr>
<td>• With metastatic colorectal cancer</td>
<td><em>NRAS</em> mutation testing to guide treatment</td>
<td>• No <em>NRAS</em> mutation testing to guide treatment</td>
<td>• Overall survival</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Disease-specific survival</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Change in disease status</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Medication use</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Resource utilization</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Treatment-related morbidity</td>
</tr>
<tr>
<td>Individuals:</td>
<td>Interventions of interest are:</td>
<td>Comparators of interest are:</td>
<td>Relevant outcomes include:</td>
</tr>
<tr>
<td>• With metastatic colorectal cancer</td>
<td><em>BRAF</em> mutation testing to guide treatment</td>
<td>• No <em>BRAF</em> mutation testing to guide treatment</td>
<td>• Overall survival</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Disease-specific survival</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Change in disease status</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Medication use</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Resource utilization</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Treatment-related morbidity</td>
</tr>
</tbody>
</table>

The epidermal growth factor receptor (EGFR) is overexpressed in colorectal cancer (CRC). EGFR-targeted therapy with monoclonal antibodies cetuximab and panitumumab has shown clear survival benefit in patients with metastatic CRC, however, this benefit depends on lack of mutations in certain genes in the signaling pathway downstream from EGFR. This review summarizes the evidence for using tumor cell *KRAS, NRAS,* and *BRAF* mutational status as a predictor of nonresponse to anti-EGFR monoclonal antibody therapy.

For individuals who have metastatic CRC who receive *KRAS* mutation 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. Mutation testing of tumor tissue performed in prospective and retrospective analyses of randomized controlled trials (RCTs) has consistently shown that the presence of a *KRAS* mutation predicts nonresponse to cetuximab.
and panitumumab, either as monotherapy or in combination with other treatment regimens, and supports the use of *KRAS* mutation analysis of tumor DNA before considering a treatment regimen. The evidence is sufficient to determine qualitatively that the technology results in a meaningful improvement in the net health outcome.

For individuals who have metastatic CRC who receive *NRAS* mutation testing to guide treatment, the evidence includes 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. Pooled analyses of *RAS* mutations beyond the common *KRAS* exon 2 mutations have been shown to predict nonresponse to cetuximab and panitumumab, and support the use of *NRAS* mutation 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 qualitatively that the technology results in a meaningful improvement in the net health outcome.

For individuals who have metastatic CRC who receive *BRAF* mutation 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 showed that anti-EGFR monoclonal antibody therapy did not improve survival in patients with *RAS* wild-type and *BRAF*-mutated tumors, however, the individual studies have been small and the results have not been consistently demonstrated in the literature. The evidence is insufficient to determine the effects of the technology on health outcomes.

**Background**

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. 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. *KRAS* mutations are found in approximately 30% to 50% of CRC tumors and are common in other tumor types. Another proto-oncogene that acts downstream from *KRAS–NRAS* harbors oncogenic mutations in codons 12, 13, or 61 that result in constitutive activation of the EGFR mediated pathway. These mutations 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 marketing approval from the U.S. Food and Drug Administration (FDA) 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 variant-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, specifically:

“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 therascreen® 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 therascreen 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.
Rationale
This evidence review was created in October 2008 and has been updated regularly with searches of the MEDLINE and EMBASE databases. The most recent literature review was performed through June 2, 2017.

Validation of the clinical use of any genetic test focuses on 3 main principles: (1) analytic validity, which refers to the technical accuracy of the test in detecting a variant that is present or in excluding a variant that is absent; (2) clinical validity, which refers to the diagnostic performance of the test (sensitivity, specificity, positive and negative predictive values) in detecting clinical disease; and (3) clinical utility (ie, how the results of the diagnostic test will be used to change management of the patient and whether these changes in management lead to clinically important improvements in health outcomes). The following is a summary of the key literature.

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 \textit{KRAS} gene do not respond to cetuximab or panitumumab therapy. More recent evidence has shown that variants in \textit{KRAS} outside exon 2, in exons 3 (codons 59 and 61) and exon 4 (codons 117 and 146), and variants in \textit{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 \textit{KRAS} exon 2 is referred to as extended \textit{RAS} testing.

\textbf{KRAS VARIANT Testing for Metastatic CRC}

\textbf{Clinical Context and Test Purpose} 
The purpose of \textit{KRAS} variant testing in individuals with metastatic CRC is to determine \textit{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 \textit{KRAS} variant testing improve health outcomes?

The following PICOTS were used to select literature to inform this review.

\textbf{Patients} 
The relevant population of interest includes individuals with metastatic CRC.

\textbf{Interventions} 
The relevant intervention of interest is \textit{KRAS} variant testing.

\textbf{Comparators} 
The relevant comparator of interest is no \textit{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.

**Analytic Validity**
Analytic validity is the technical accuracy of the test in detecting a variant that is present, or in excluding a variant that is absent. The Food and Drug Administration (FDA) Summary of Safety and Effectiveness Data documents have described the analytic validity of the therascreen KRAS RGQ PCR Kit and cobas KRAS Mutation Test, which were approved through the premarket approval process. Both FDA-approved tests are real-time polymerase chain reaction (PCR) tests intended to detect somatic variants in KRAS genes and have documented analytic validity including concordance studies between the test and Sanger sequencing, the limits of detection for both variants detected, cross-reactivity to interference by substances such as hemoglobin, albumin, and blood preservatives, and reproducibility. These results have shown that both tests have high analytic sensitivity and specificity compared with Sanger sequencing, high reproducibility, sufficient levels of detection and limits of blank (ie, highest analyte concentration expected to be detected when blank samples are tested), and low cross-reactivity. No published studies are available demonstrating the analytic validity of laboratory-developed tests (LDTs) for KRAS variants.

**Section Summary: Analytic Validity**
Evidence for the analytic validity of the therascreen KRAS RGQ PCR Kit and cobas KRAS Mutation Test include FDA Summary of Safety and Effectiveness Data for both tests. There is a lack of published evidence on the analytic validity of LDTs to detect KRAS variants. However, it is expected that the analytic validity will be high when testing is performed according to optimal laboratory standards.

**Clinical Validity**
This evidence review has been informed, in part, by a 2008 TEC Assessment. Additional evidence derives from 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. The original 2007 study, designed as a multicenter RCT, was not blinded because of expected skin toxicity related to panitumumab administration. 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 (FFPE) tumor sections and a validated KRAS variant kit (DxS) that identifies 7 somatic variants located in codons 12 and 13 using real-time PCR. 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>
<thead>
<tr>
<th>Outcomes</th>
<th>KRAS WT (n=243 [57%])</th>
<th>KRAS MT (n=184 [43%])</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P (n=124)</td>
<td>BSC (n=119)</td>
</tr>
<tr>
<td>Median progression-free survival, wk</td>
<td>12.3</td>
<td>7.3</td>
</tr>
<tr>
<td>Hazard ratio (95% CI)</td>
<td>0.45 (0.34 to 0.59)</td>
<td>0.99 (0.73 to 1.36)</td>
</tr>
<tr>
<td>Response rate, %</td>
<td>17</td>
<td>0</td>
</tr>
</tbody>
</table>
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 of 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 according to KRAS variant status was performed by Van Cutsem et al (2009). 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 FFPE 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 according to 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)**

<table>
<thead>
<tr>
<th>Outcomes</th>
<th>ITT&lt;sup&gt;a&lt;/sup&gt;</th>
<th>KRAS WT (n=348 [64%]&lt;sup&gt;b&lt;/sup&gt;)</th>
<th>KRAS MT (n=192 [36%]&lt;sup&gt;b&lt;/sup&gt;)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C+F</td>
<td>F</td>
<td>C+F</td>
</tr>
<tr>
<td>n</td>
<td>599</td>
<td>599</td>
<td>172</td>
</tr>
<tr>
<td>RR (95% CI), %</td>
<td>46.9 (42.9 to 50.1)</td>
<td>38.7 (34.8 to 42.8)</td>
<td>59.3 (51.6 to 66.7)</td>
</tr>
<tr>
<td>mPFS, mo&lt;sup&gt;c&lt;/sup&gt;</td>
<td>8.9</td>
<td>8.0</td>
<td>9.9</td>
</tr>
</tbody>
</table>

Adapted from Amado et al (2008).
In a third trial, the randomized, phase 2 OPUS trial, the intention-to-treat (ITT) population consisted of 337 patients randomized to cetuximab and folinic acid (leucovorin), 5-flourouracil, and oxaliplatin (FOLFOX) or to FOLFOX alone in the first-line treatment of metastatic CRC. 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 the groups. The 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. The study findings are summarized in Table 3.

### 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)

<table>
<thead>
<tr>
<th>Outcomes</th>
<th>KRAS WT (n=134 [58%])</th>
<th>KRAS MT (n=99 [42%])</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C+Fx</td>
<td>Fx</td>
</tr>
<tr>
<td>n (KRAS-</td>
<td>61</td>
<td>73</td>
</tr>
<tr>
<td>evaluable)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RR (95% CI),</td>
<td>60.7 (47.3 to 72.9)</td>
<td>37.0 (26.0 to 49.1)</td>
</tr>
<tr>
<td>%</td>
<td>0.011</td>
<td>0.106</td>
</tr>
<tr>
<td>Odds ratio</td>
<td>2.54 (1.24 to 5.23)</td>
<td>0.51 (0.22 to 1.15)</td>
</tr>
<tr>
<td>(95% CI)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>mPFS, moa</td>
<td>7.7</td>
<td>7.2</td>
</tr>
<tr>
<td>p</td>
<td>0.016</td>
<td>0.019</td>
</tr>
<tr>
<td>Hazard ratio</td>
<td>0.57</td>
<td>1.83</td>
</tr>
</tbody>
</table>

Adapted from Bokemeyer et al (2009).

C: cetuximab; CI: confidence interval; Fx: FOLFOX; MT: mutated; mPFS: median progression-free survival; RR: response rate; WT: wild-type.

*Confidence intervals for mPFS 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 regimen plus cetuximab (CBC regimen, n=377). KRAS variant was found in 40% of tumors (108 from patients in the CB group, 98 from the CBC 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 the 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. 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 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. 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. An updated analysis of CRYSTAL (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. Subsequent to the initial published analysis, which had 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 FORFIRI alone as a second-line treatment. The study 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 was 14.5 months and 12.5 months, respectively (HR=0.85, 95% CI, 0.70 to 1.04; p=0.12); response rates were improved to 35% and 10%, respectively, with the addition of panitumumab. In patients with mutated KRAS, no difference was reported in efficacy. Adverse events were comparable across arms. The authors 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). 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. Analysis was by ITT and treatment allocation was not masked. Further analysis of other variants, including BRAF, was done; 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 KRAS variant status and 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. The overall KRAS variant rate was 38% (829/2188 patients). The results of the meta-analysis 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. 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.

A 2012 pooled analysis of wild-type KRAS tumors from the CRYSTAL and OPUS trial data assessed extended survival data and enhancement in the ascertainment rate of KRAS and BRAF tumor variant status. 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. Treatment arms were compared by variant status using log-rank and Cochran-Mantel-Haenszel tests. In 845 patients with wild-type KRAS tumors, adding cetuximab to chemotherapy led to a 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, 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 (Cetuximab or Panitumumab)**

In addition to the 3 randomized trials discussed here, a number of single-arm studies have retrospectively evaluated KRAS variant status and treatment response in patients with metastatic CRC. Overall they have shown similar nonresponse rates to anti-EGFR monoclonal antibodies in patients with mutated KRAS tumors. Two of these single-arm studies have also reported differences in PFS and OS.

**Section Summary: Clinical Validity**

Evidence for the clinical validity 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.

**Clinical Utility**

Cetuximab and panitumumab are anti-EGFR monoclonal antibodies indicated 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: Clinical Utility**

Direct evidence for the clinical utility of KRAS variant testing includes RCTs. RCTs supporting Food and Drug Administration approvals for cetuximab and panitumumab has 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 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 relevant intervention of interest is NRAS variant testing.

Comparators
The relevant comparator of interest is 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.

Analytic Validity
No published studies are available demonstrating the analytic validity of LDTs for NRAS variants in CRC samples.

Section Summary: Analytic Validity
There is a lack of published evidence on the analytic validity of LDTs to detect NRAS variants in CRC samples. However, it is expected that analytic validity will be high when testing is performed according to optimal laboratory standards.

Clinical Validity

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.

In 2015, Peeters et al 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. 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. The PFS and OS HRs for panitumumab plus FOLFIRI vs FOLIRI alone are summarized in Table 4. 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

<table>
<thead>
<tr>
<th>RAS Status</th>
<th>PFS HR (95% CI)</th>
<th>P</th>
<th>OS HR (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild-type RAS</td>
<td>0.70 (0.54 to 0.94)</td>
<td>0.007</td>
<td>0.81 (0.63 to 0.99)</td>
<td>0.08</td>
</tr>
</tbody>
</table>
For RAS wild-type patients, the ORR was 41% when they were treated with panitumumab plus FOLFIRI vs 10% when treated 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.

In 2015, Van Cutsem et al 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. 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<.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 (PRIME) RCT. A total of 108 (17%) of 641 tumor specimens that did not harbor KRAS variants in exon 2 had variants in other RAS exons, including NRAS (exons 2 or 4) and KRAS (exons 3 and 4). For patients with 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 do not discriminate specific types of RAS variants, so it is not possible to relate NRAS to these results. Furthermore, the numbers of patients involved are very small, further limiting conclusions.

Tumor specimens (288 of 320) from a 2007 RCT were analyzed using massively parallel multigene sequencing (next-generation sequencing) to investigate whether EGFR pathway variants would predict response to monotherapy with panitumumab compared with best supportive care. 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 2010 retrospective consortium analysis reported results of centrally performed high-throughput mass spectrometric variant profiling of CRC specimens gathered from 11 centers in 7 European countries. 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 previously discussed was also shown in 2010 a study 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. 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.

A 2014 systematic review evaluated the predictive value of NRAS variants on clinical outcomes of anti-EGFR therapy in CRC. The meta-analysis included data from 3 studies included in this evidence review. Reviewers suggested that the pooled analyses showed a trend toward a poor odds ratio (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.

**Section Summary: Clinical Validity**

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 variants in NRAS are predictive of nonresponse. However, because of the low prevalence of NRAS variants, the predictive value of NRAS variants is uncertain.

**Clinical Utility**
Documentation of KRAS wild-type status is required prior to treatment with cetuximab or panitumumab. 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: Clinical Utility**
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 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 management decisions.

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 relevant intervention of interest is BRAF variant testing.

**Comparators**
The relevant comparator of interest is 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.

**Analytic Validity**
No published studies are available demonstrating the analytic validity of LDTs for \( BRAF \) variants in CRC samples.

**Section Summary: Analytic Validity**
There is a lack of published evidence on the analytic validity of LDTs to detect \( BRAF \) variants in CRC samples. However, it is expected that analytic validity will be high when testing is performed according to optimal laboratory standards.

**Clinical Validity**

**Systematic Reviews**
A 2015 meta-analysis identified 9 phase 3 trials that compared cetuximab or panitumumab with standard therapy or best supportive care.\(^32\) 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 2011 meta-analysis of \( BRAF \) variants and resistance to anti-EGFR monoclonal antibodies in patients with metastatic CRC was performed.\(^33\) 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.\(^34-\)\(^43\) All were conducted retrospectively (1 study was a nonconcurrent analysis of response in a population previously randomized\(^43\)). 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, \( KRAS \) variant status was unknown) 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 \( BRAF \) variant was 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 results of the meta-analysis using homogenous metastatic CRC patients with assessors blinded to the clinical data.
Mao’s meta-analysis (2011) also assessed \textit{BRAF} V600E variant and resistance to anti-EGFR monoclonal antibodies in patients with metastatic CRC.\textsuperscript{33} The same 11 studies were selected. Seven included unselected patients, and 4 studies included only patients with wild-type \textit{KRAS}. The primary end point was ORR. In the 7 studies with unselected patients, \textit{BRAF} variant status was performed successfully on 546 metastatic CRC. \textit{BRAF} variants were detected in 8.8\% of primary tumors. The ORR of metastatic CRC patients with mutant \textit{BRAF} was 29.2\% and 33.5\% in patients with wild-type \textit{BRAF}. In the 4 studies that included patients with wild-type \textit{KRAS}, \textit{BRAF} variant status was performed successfully on 376 wild-type \textit{KRAS} metastatic CRC. \textit{BRAF} variants were detected in 10.6\% of primary tumors. The ORR of patients with mutant \textit{BRAF} genes was 0.0\%, whereas it was 36.3\% in patients with wild-type. Reviewers concluded that their results provided evidence that the \textit{BRAF} variant is associated with lack of response in wild-type \textit{KRAS} metastatic CRC treated with anti-EGFR monoclonal antibodies.

\textbf{Retrospective Studies}

Di Nicolantonio et al (2008) retrospectively analyzed 113 patients with metastatic CRC who had received cetuximab or panitumumab.\textsuperscript{35} None of the \textit{BRAF}-mutated tumors (0/11) responded to treatment, whereas 32.4\% (22/68) of the wild-type \textit{BRAF} did. Loupakis et al (2009) retrospectively assessed 87 patients receiving irinotecan and cetuximab.\textsuperscript{38} Of the 87 patients in the study, \textit{BRAF} was mutated in 13 patients, and none of whom responded to chemotherapy, compared with 32\% (24/74) of patients with wild-type \textit{BRAF} who did. In the CAIRO2 study (2009), retrospective analysis of \textit{BRAF} variants was performed in 516 available tumors from patients previously randomized to the CB regimen or to the CBC regimen.\textsuperscript{43} A \textit{BRAF} variant was found in 8.7\% (n=45) of the tumors. Patients with a \textit{BRAF} variant had a shorter median PFS and OS compared with wild-type \textit{BRAF} tumors in both treatment arms. The authors concluded that a \textit{BRAF} variant was a negative prognostic marker in patients with metastatic CRC and that this effect, in contrast with \textit{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.\textsuperscript{9} A 2014 analysis of \textit{BRAF} variants in this patient population and the influence of \textit{BRAF} variant status showed that, for the wild-type \textit{KRAS} and \textit{BRAF}-mutated patients, OS for cetuximab plus FOLFIRI and FOLFIRI alone was 14.1 months and 10.3 months, respectively (p=0.744).\textsuperscript{44} Although this difference was not statistically significant, it showed a trend toward improved OS, PFS, and response, suggesting that wild-type \textit{KRAS}- and \textit{BRAF}-mutant patients might benefit from anti-EGFR therapy.

De Roock et al (2010) reported on the effects of 4 variants, including \textit{BRAF}, on the efficacy of cetuximab and chemotherapy in chemotherapy-refractory metastatic CRC in 773 primary tumor samples.\textsuperscript{29} Tumor samples were from fresh frozen or FFPE tissue, and the variant status was compared with retrospectively collected clinical outcomes including ORR, PFS, and OS. \textit{BRAF} variants were found in 36 (4.7\%) of 761 tumors. In patients with wild-type \textit{KRAS}, carriers of \textit{BRAF} variants had a significantly lower response rate (8.3\% [2/24] patients) than wild-type \textit{BRAF} (38.0\% [124/326] patients; OR=0.15; 95\% CI, 0.02 to 0.51; p=0.001). PFS for \textit{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).

An updated analysis of the CRYSTAL trial (2011) reported on longer follow-up and larger numbers of 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. 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: Clinical Validity**

Evidence for the clinical validity 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.

**Clinical Utility**

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: Clinical Utility**

Direct evidence for the clinical utility 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 management decisions, 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-epidermal growth factor receptor monoclonal antibody therapy did not improve survival in patients with RAS wild-type and 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**

**Objective**
In 2017, clinical input was sought to help determine whether testing for BRAF V600E variant status for individuals with metastatic colorectal cancer would provide a meaningful clinical benefit, defined as avoidance of anti-epidermal growth factor receptor (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.

**Respondents**
Clinical input was provided by the following specialty societies and physician members identified by a specialty society or clinical health system:

- Association for Molecular Pathology (AMP)
- Carmen J. Allegra, MD, Medical Oncology; identified by American Society of Clinical Oncology (ASCO)
- Christopher H. Lieu, MD, Medical Oncology; identified by American Society of Clinical Oncology (ASCO)
Clinical Input Responses

Clinical Indication: Testing for BRAF V600E variant status for individuals with metastatic colorectal cancer to guide treatment with EGFR-targeted therapy

<table>
<thead>
<tr>
<th>Respondent</th>
<th>Specialty</th>
<th>Identified by</th>
</tr>
</thead>
<tbody>
<tr>
<td>Association for Molecular Pathology</td>
<td>Molecular Pathology</td>
<td></td>
</tr>
<tr>
<td>Dr. Alegra</td>
<td>Medical Oncology</td>
<td>American Society of Clinical Oncology</td>
</tr>
<tr>
<td>Dr. Liu</td>
<td>Medical Oncology</td>
<td>American Society of Clinical Oncology</td>
</tr>
<tr>
<td>Dr. Smaglo and Dr. Chandar</td>
<td>Gastrointestinal Oncology and Hematology/Oncology</td>
<td>Baylor College of Medicine</td>
</tr>
<tr>
<td>Anonymous</td>
<td>Medical Oncology</td>
<td>Catholic Health Initiatives</td>
</tr>
<tr>
<td>Anonymous</td>
<td>Medical Oncology</td>
<td>Cancer Treatment Centers of America</td>
</tr>
<tr>
<td>Dr. Meiri</td>
<td>Medical Oncology</td>
<td>Cancer Treatment Centers of America</td>
</tr>
<tr>
<td>Dr. Loaiza-Bonilla</td>
<td>Medical Oncology</td>
<td>Cancer Treatment Centers of America</td>
</tr>
<tr>
<td>Anonymous</td>
<td>Pathology and Laboratory Medicine</td>
<td>Cancer Treatment Centers of America</td>
</tr>
<tr>
<td>Dr. Chowdhury</td>
<td>Medical Oncology</td>
<td>Cancer Treatment Centers of America</td>
</tr>
</tbody>
</table>

Confidence Level that Clinical Use Expected to Provide Meaningful Clinical Benefit:

<table>
<thead>
<tr>
<th>Respondent</th>
<th>Specialty</th>
<th>Identified by</th>
</tr>
</thead>
<tbody>
<tr>
<td>Association for Molecular Pathology</td>
<td>Molecular Pathology</td>
<td></td>
</tr>
<tr>
<td>Dr. Alegra</td>
<td>Medical Oncology</td>
<td>American Society of Clinical Oncology</td>
</tr>
<tr>
<td>Dr. Liu</td>
<td>Medical Oncology</td>
<td>American Society of Clinical Oncology</td>
</tr>
<tr>
<td>Dr. Smaglo and Dr. Chandar</td>
<td>Gastrointestinal Oncology and Hematology/Oncology</td>
<td>Baylor College of Medicine</td>
</tr>
<tr>
<td>Anonymous</td>
<td>Medical Oncology</td>
<td>Catholic Health Initiatives</td>
</tr>
<tr>
<td>Anonymous</td>
<td>Medical Oncology</td>
<td>Cancer Treatment Centers of America</td>
</tr>
<tr>
<td>Dr. Meiri</td>
<td>Medical Oncology</td>
<td>Cancer Treatment Centers of America</td>
</tr>
<tr>
<td>Dr. Loaiza-Bonilla</td>
<td>Medical Oncology</td>
<td>Cancer Treatment Centers of America</td>
</tr>
<tr>
<td>Anonymous</td>
<td>Pathology and Laboratory Medicine</td>
<td>Cancer Treatment Centers of America</td>
</tr>
<tr>
<td>Dr. Chowdhury</td>
<td>Medical Oncology</td>
<td>Cancer Treatment Centers of America</td>
</tr>
</tbody>
</table>

Confidence Level that Clinical Use is Consistent with Generally Accepted Medical Practice:

<table>
<thead>
<tr>
<th>Respondent</th>
<th>Specialty</th>
<th>Identified by</th>
</tr>
</thead>
<tbody>
<tr>
<td>Association for Molecular Pathology</td>
<td>Molecular Pathology</td>
<td></td>
</tr>
<tr>
<td>Dr. Alegra</td>
<td>Medical Oncology</td>
<td>American Society of Clinical Oncology</td>
</tr>
<tr>
<td>Dr. Liu</td>
<td>Medical Oncology</td>
<td>American Society of Clinical Oncology</td>
</tr>
<tr>
<td>Dr. Smaglo and Dr. Chandar</td>
<td>Gastrointestinal Oncology and Hematology/Oncology</td>
<td>Baylor College of Medicine</td>
</tr>
<tr>
<td>Anonymous</td>
<td>Medical Oncology</td>
<td>Catholic Health Initiatives</td>
</tr>
<tr>
<td>Anonymous</td>
<td>Medical Oncology</td>
<td>Cancer Treatment Centers of America</td>
</tr>
<tr>
<td>Dr. Meiri</td>
<td>Medical Oncology</td>
<td>Cancer Treatment Centers of America</td>
</tr>
<tr>
<td>Dr. Loaiza-Bonilla</td>
<td>Medical Oncology</td>
<td>Cancer Treatment Centers of America</td>
</tr>
<tr>
<td>Anonymous</td>
<td>Pathology and Laboratory Medicine</td>
<td>Cancer Treatment Centers of America</td>
</tr>
<tr>
<td>Dr. Chowdhury</td>
<td>Medical Oncology</td>
<td>Cancer Treatment Centers of America</td>
</tr>
</tbody>
</table>

Additional Comments
“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 acid (leucovorin calcium), 5-fluorouracil, irinotecan
hydrochloride, and oxaliplatin] as first-line therapy, followed by enrollment in a clinical trial.” (Association for Molecular Pathology [AMP])

- “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.” (Association for Molecular Pathology [AMP])

- “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. 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.” (Drs. Smaglo and Chandar, identified by Dan L Duncan Comprehensive Cancer Center, Baylor College of Medicine)

- “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... 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.” (Eyal Meiri, MD, [CTCA])

See Appendices 1 and 2 for details of the clinical input.

Supplemental Information

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.2.2017). 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. The guidelines also state that the presence of the BRAF V600E variant makes response to panitumumab and cetuximab highly unlikely.

**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*, has stated that evidence is insufficient to support the clinical validity or utility of testing colorectal cancer specimens for NRAS variants to guide patient management. That same review further 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.

### Table 5. Summary of Recommendations

<table>
<thead>
<tr>
<th>Guideline Statements</th>
<th>Type</th>
<th>SOE</th>
<th>QOE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colorectal carcinoma patients being considered for anti-EGFR therapy</td>
<td>Recommendation</td>
<td>Convincing/adequate, benefits</td>
<td>High/intermediate</td>
</tr>
</tbody>
</table>
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) |  

outweigh harms

| 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

| 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. 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.
**Medicare National Coverage**

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

**Ongoing and Unpublished Clinical Trials**

Some currently unpublished trials that might influence this review are listed in Table 5.

### Table 6. Summary of Key Trials

<table>
<thead>
<tr>
<th>NCT No.</th>
<th>Trial Name</th>
<th>Planned Enrollment</th>
<th>Completion Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ongoing</td>
<td>Treatment Strategies in Colorectal Cancer Patients With Initially Unresectable Liver-only Metastases (CAIRO5)</td>
<td>640</td>
<td>Jul 2025</td>
</tr>
</tbody>
</table>

NCT: national clinical trial.

**References**


Billing Coding/Physician Documentation Information

81210  BRAF (v-raf murine sarcoma viral oncogene homolog B1) (eg, colon cancer), gene analysis, V600E variant
81275  KRAS (v-Ki-ras2 Kirsten rat sarcoma viral oncogene) (eg, carcinoma) gene analysis, variants in codons 12 and 13
81276  KRAS (Kirsten rat sarcoma viral oncogene homolog) (eg, carcinoma) gene analysis; additional variant(s) (eg, codon 61, codon 146) (new code 1/1/2016)
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) (new code 1/1/2016)
81403  Molecular pathology procedure, Level 4 (eg, analysis of single exon by DNA sequence analysis, analysis of > 10 amplicons using multiplex PCR in 2 or more independent reactions, mutation scanning or duplication/deletion variants of 2-5 exons) ABL1 (c-abl oncogene 1, receptor tyrosine kinase) (eg, acquired imatinib tyrosine kinase inhibitor resistance), variants in the kinase domain DAZ/SRY (deleted in azoospermia and sex determining region Y) (eg, male infertility), common deletions (eg, AZFa, AZFb, AZFc, AZFd) GJB1 (gap junction protein, beta 1) (eg, Charcot-Marie-Tooth X-linked), full gene sequence JAK2 (Janus kinase 2) (eg, myeloproliferative disorder), exon 12 sequence and exon 13 sequence, if performed KRAS (v-Ki-ras2 Kirsten rat sarcoma viral oncogene) (eg, carcinoma), gene analysis, variant(s) in exon 2 MPL (myeloproliferative leukemia virus oncogene, thrombopoietin receptor, TPOR) (eg, myeloproliferative disorder), exon 10 sequence VHL (von Hippel-Lindau tumor suppressor) (eg, von Hippel-Lindau familial cancer syndrome), deletion/duplication analysis VWF (von Willebrand factor) (eg, von Willebrand disease types 2A, 2B, 2M), targeted sequence analysis (eg, exon 28)
81404  Molecular pathology procedure, Level 5 (eg, analysis of 2-5 exons by DNA sequence analysis, mutation scanning or duplication/deletion variants of 6-10 exons, or characterization of a dynamic mutation disorder/triplet repeat by Southern blot analysis)
88363  Examination and selection of retrieved archival (ie, previously diagnosed) tissue(s) for molecular analysis (eg, KRAS mutational analysis)

ICD10 Codes

C18.0-  Malignant neoplasm of colon code range
C18.9  Malignant neoplasm of rectosigmoid junction
C19   Malignant neoplasm of rectum
C20   Malignant neoplasm of rectum
C78.5  Secondary malignant neoplasm of large intestine and rectum

Additional Policy Key Words

N/A
Policy Implementation/Update Information

1/1/11 New policy; may be considered medically necessary
5/1/11 No policy statement changes.
5/1/12 Title changed to indicate inclusion of BRAF testing to the policy; BRAF testing policy statement added as investigational to predict nonresponse to anti-EGFR monoclonal antibodies cetuximab and panitumumab in the treatment of metastatic colorectal cancer; KRAS policy statement unchanged.
5/1/13 No policy statement changes.
5/1/14 No policy statement changes.
5/1/15 Title changed to indicate inclusion of NRAS testing to the policy; NRAS testing policy statement added as investigational to predict nonresponse to anti-EGFR monoclonal antibodies cetuximab and panitumumab in the treatment of metastatic colorectal cancer. KRAS testing not meeting criteria changed from not medically necessary to investigational.
5/1/16 No policy statement changes.
12/1/16 Policy statement revised to indicate that NRAS testing policy statement added as medically necessary to predict nonresponse to anti-EGFR monoclonal antibodies cetuximab and panitumumab in the treatment of metastatic colorectal cancer.
5/1/17 No policy statement changes.
5/1/18 BRAF variant analysis 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. Title changed to “KRAS, NRAS, and BRAF Variant Analysis in Metastatic Colorectal Cancer”. Removed BRAF mutation analysis is considered investigational to predict nonresponse to anti-EGFR monoclonal antibodies cetuximab and panitumumab in the treatment of metastatic colorectal cancer.

State and Federal mandates and health plan contract language, including specific provisions/exclusions, take precedence over Medical Policy and must be considered first in determining eligibility for coverage. The medical policies contained herein are for informational purposes. The medical policies do not constitute medical advice or medical care. Treating health care providers are independent contractors and are neither employees nor agents Blue KC and are solely responsible for diagnosis, treatment and medical advice. No part of this publication may be reproduced, stored in a retrieval system or transmitted, in any form or by any means, electronic, photocopying, or otherwise, without permission from Blue KC.

APPENDIX

Appendix Table 1. Categories of Genetic Testing Addressed in 2.04.53

<table>
<thead>
<tr>
<th>Category</th>
<th>Addressed</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Testing of an affected individual’s germline to benefit the individual</td>
<td></td>
</tr>
<tr>
<td>1a. Diagnostic</td>
<td></td>
</tr>
<tr>
<td>1b. Prognostic</td>
<td></td>
</tr>
<tr>
<td>1c. Therapeutic</td>
<td></td>
</tr>
<tr>
<td>2. Testing cancer cells from an affected individual to benefit the individual</td>
<td></td>
</tr>
</tbody>
</table>
2a. Diagnostic
2b. Prognostic
2c. Therapeutic

3. Testing an asymptomatic individual to determine future risk of disease
4. Testing of an affected individual's germline to benefit family members
5. Reproductive testing
   5a. Carrier testing: preconception
   5b. Carrier testing: prenatal
   5c. In utero testing: aneuploidy
   5d. In utero testing: familial variants
   5e. In utero testing: other
   5f. Preimplantation testing with in vitro fertilization

APPENDIX 1: CLINICAL INPUT

Appendix Table 2. Respondent Profile

<table>
<thead>
<tr>
<th>No.</th>
<th>Name of Organization</th>
<th>Specialty Society</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Association for Molecular Pathology</td>
<td>Molecular Pathology</td>
</tr>
</tbody>
</table>

**Physician**

<table>
<thead>
<tr>
<th>No.</th>
<th>Name</th>
<th>Degree</th>
<th>Institutional Affiliation</th>
<th>Clinical Specialty</th>
<th>Board Certification and Fellowship Training</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>Anonymous</td>
<td>MD</td>
<td>Cancer Treatment Centers of America (CTCA)</td>
<td>Medical Oncologist</td>
<td>Internal Medicine and Medical Oncology</td>
</tr>
<tr>
<td>3</td>
<td>Eyal Meiri</td>
<td>MD</td>
<td>Cancer Treatment Centers of America (CTCA)</td>
<td>Medical Oncology</td>
<td>Medical Oncology</td>
</tr>
<tr>
<td>4</td>
<td>Arturo Loaiza-Bonilla</td>
<td>MD, MSEd</td>
<td>Cancer Treatment Centers of America (CTCA)</td>
<td>Medical Oncology, Gastrointestinal Oncology</td>
<td>ABIM certified in Internal Medicine, Medical Oncology and Hematology. Fellowship.</td>
</tr>
<tr>
<td>5</td>
<td>Anonymous</td>
<td>MD</td>
<td>Cancer Treatment Centers of America (CTCA)</td>
<td>Medical Oncology Pathology and Laboratory Medicine</td>
<td>American Board of Pathology</td>
</tr>
<tr>
<td>6</td>
<td>Shahin Chowdhury</td>
<td>DO</td>
<td>Cancer Treatment Centers of America (SERMC)</td>
<td>Medical Oncology</td>
<td>American College of Osteopathic Internists (ACOI)</td>
</tr>
</tbody>
</table>

Identified by Cancer Treatment Centers of America (CTCA)

Identified by Dan L Duncan Comprehensive Cancer Center, Baylor College of Medicine

<table>
<thead>
<tr>
<th>No.</th>
<th>Name</th>
<th>Degree</th>
<th>Institutional Affiliation</th>
<th>Clinical Specialty</th>
<th>Board Certification and Fellowship Training</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Manisha Chandar</td>
<td>DO</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Identified by American Society of Clinical Oncology

<table>
<thead>
<tr>
<th>No.</th>
<th>Name</th>
<th>Degree</th>
<th>Institutional Affiliation</th>
<th>Clinical Specialty</th>
<th>Board Certification and Fellowship Training</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>Carmen J. Allegra</td>
<td>MD</td>
<td>University of Florida</td>
<td>Medical Oncology</td>
<td>Internal Medicine and Oncology</td>
</tr>
<tr>
<td>9</td>
<td>Christopher H. Lieu</td>
<td>MD</td>
<td>University of Colorado</td>
<td>Medical Oncology</td>
<td>Medical Oncology - Fellowship Training - MD Anderson Cancer Center</td>
</tr>
</tbody>
</table>

Identified by Catholic Health Initiatives (CHI)

<table>
<thead>
<tr>
<th>No.</th>
<th>Name</th>
<th>Degree</th>
<th>Institutional Affiliation</th>
<th>Clinical Specialty</th>
<th>Board Certification and Fellowship Training</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>Anonymous</td>
<td>MD</td>
<td></td>
<td>Medical Oncology</td>
<td>Medical Oncology and Internal Medicine</td>
</tr>
</tbody>
</table>

Appendix Table 3. Respondent Conflict of Interest Disclosure

<table>
<thead>
<tr>
<th>No.</th>
<th>1. Research support related to the topic where clinical input is being sought</th>
<th>2. Positions, paid or unpaid, related to the topic where clinical input is being sought</th>
<th>3. Reportable, more than $1,000, health care–related assets or sources of income for myself, my spouse, or my dependent children related to the topic where clinical input is being sought</th>
<th>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</th>
<th>Yes/No</th>
<th>Explanation</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Explanation</td>
</tr>
<tr>
<td>3</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Explanation</td>
</tr>
<tr>
<td>4</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Explanation</td>
</tr>
</tbody>
</table>
APPENDIX 2: CLINICAL INPUT RESPONSES

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.

<table>
<thead>
<tr>
<th>Populations</th>
<th>Interventions</th>
<th>Comparators</th>
<th>Outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Individuals:</td>
<td>Interventions of interest are:</td>
<td>Comparators of interest are:</td>
<td>Relevant outcomes include:</td>
</tr>
<tr>
<td>• With metastatic colorectal cancer</td>
<td>• BRAF variant testing to guide treatment</td>
<td>• No BRAF variant testing to guide treatment</td>
<td>• Overall survival</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Disease-specific survival</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Change in disease status</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Medication use</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Treatment-related morbidity</td>
</tr>
</tbody>
</table>

Clinical input is sought to help determine whether testing for BRAF V600E variant status for individuals with metastatic colorectal cancer 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
1. 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 colorectal cancer 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.

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
bimarker 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 have a BRAF mutation have significantly poorer outcomes as assessed 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 these 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 a particularly poor prognosis. It is important to know that 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 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 Evidence Summary 2.04.08 “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 V600E 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 analyses 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].


5. Patients with metastatic colorectal cancer who have been shown to 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 one in 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 the adjuvant setting.

Studies using the FDA-approved and newer developed LDT tests have found adequate evidence that KRAS mutation analysis reliably and accurately detects common BRAF mutations. Results from RT-PCR testing are comparable to next gen sequencing. Testing using immunohistochemical stain (clone VE1) for BRAF V600E in 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.


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 is not a perfect biomarker or prognosis) since I reserve anti-EGFR therapy for 2nd line therapy.

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. 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.


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 bevocizumab versus FOLFI RI 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.

There is evidence that patients with metastatic colorectal cancer with BRAF V600E variants do not benefit from treatment with anti-EGFR therapy. While BRAF V600E is a known prognostic factor, we suggest a lack of benefit of standard therapy (chemo + EGFR inhibition).


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.

- BRAF mutations associated with low probability response to epidermal growth factor receptor (EGFR) inhibitors.
- BRAF V600E associated with worse prognosis.
  - High microsatellite instability (MSI), could be candidate for immunotherapy.
- Non V600E BRAF associated with better prognosis. All these are important for prognosis and treatment of patients with colorectal cancer.

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

<table>
<thead>
<tr>
<th>No.</th>
<th>Yes / No</th>
<th>Low</th>
<th>Intermediate</th>
<th>High</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Confidence</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Response</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Use the 1 to 5 scale outlined below to indicate your level of confidence that there is adequate evidence that supports your conclusions.</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
3. Based on the evidence and your clinical experience for \textit{BRAF} V600E variant testing to guide treatment with \textit{EGFR}-targeted therapy in individuals with metastatic colorectal cancer:

a. Respond YES or NO for each indication whether this intervention is consistent with generally accepted medical practice.

b. Use the 1 to 5 scale outlined below to indicate your level of confidence in your conclusions.

<table>
<thead>
<tr>
<th>No.</th>
<th>Yes / No</th>
<th>Low Confidence</th>
<th>Intermediate Confidence</th>
<th>High Confidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Yes</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Yes</td>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Yes</td>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Yes</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Yes</td>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>No</td>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Yes</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Yes</td>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Yes</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Yes</td>
<td>5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

4. Additional comments and/or any citations supporting your clinical input on the use of \textit{BRAF} V600E variant testing to guide treatment with \textit{EGFR}-targeted therapy in individuals with metastatic colorectal cancer.

<table>
<thead>
<tr>
<th>No.</th>
<th>Additional Comments</th>
</tr>
</thead>
</table>
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 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:


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 under way. 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.


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.

I don’t use BRAF test to determine use of anti-EGFR therapy.

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.


Is there any evidence missing from the attached draft review of evidence that demonstrates clinical benefit?
KRAS, NRAS, and BRAF Variant Analysis in Metastatic Colorectal Cancer 2.04.53

<table>
<thead>
<tr>
<th>No.</th>
<th>Yes/No</th>
<th>Citations of Missing Evidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Yes</td>
<td>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. <a href="http://dx.doi.org/10.1016/j.jmoldx.2016.11.001">Link</a></td>
</tr>
</tbody>
</table>
| 3   | No     | 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 RQ 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 they 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:  
| 4   | Yes    | Further, the evidence summary lists two FDA-approved tests for KRAS variant analysis, the Cobas KRAS mutation test and the therascreen KRAS RQ 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 RQ PCR kits do not detect all the variants for KRAS and NRAS recommended by current guidelines. [Link](https://www.fda.gov/drugs/informationondrugs/approveddrugs/ucm565785.htm) |
| 5   | No     | 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 increased in 18% of the experimental arm and 8% of the control arm. Almost 50% of patients in the metastatic setting were presented at ESMO 2017 in September. There was a 41% confirmed ORR increase in 9.3 months). [2] 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] |
| 6   | No     | Drug Information: [Link](https://www.fda.gov/drugs/informationondrugs/approveddrugs/ucm565785.htm)  
  - Huijberts S, Schellens JHM, Fahk M, et al. BEACON CRC (binimetinib [BINI], encorafenib [ENCE], 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/leucovorin 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). |
| 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) could be beneficial in patients with metastatic colorectal cancer with known BRAF V600E mutations. [3] |

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.


<table>
<thead>
<tr>
<th>No.</th>
<th>Yes/No</th>
<th>Citations of Missing Evidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>Yes</td>
<td>As noted above in responses to #1 and 4</td>
</tr>
<tr>
<td>9</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>NR</td>
<td></td>
</tr>
</tbody>
</table>

NR: no response.