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

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### DESCRIPTION

The epidermal growth factor receptor (EGFR) is overexpressed in colorectal cancer (CRC). EGFR-targeted therapy combined with monoclonal antibodies cetuximab and panitumumab has shown a clear survival benefit in patients with metastatic CRC. However, this benefit depends on a lack of variants in certain genes in the signaling pathway downstream from the EGFR. It has been hypothesized that knowledge of tumor cell KRAS, NRAS, and BRAF variant status might be used to predict nonresponse to anti-EGFR monoclonal antibody therapy. Typically, the evaluation of RAS mutation status requires tissue biopsy. Circulating tumor DNA or circulating tumor cell testing (also known as liquid biopsy) is proposed as a noninvasive alternative.

### OBJECTIVE

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

### BACKGROUND

Cetuximab (Erbitux, ImClone Systems) and panitumumab (Vectibix, Amgen) are monoclonal antibodies that bind to the EGFR, preventing intrinsic ligand binding and activation of downstream signaling pathways vital for cancer cell proliferation, invasion, metastasis, and stimulation of neovascularization.

The RAS-RAF-MAP kinase pathway is activated in the EGFR cascade. RAS proteins are G proteins that cycle between active (RAS-GTP) and inactive (RAS-GDP) forms, in response to stimulation from a cell surface receptor such as EGFR, and act as a binary switch between the cell surface EGFR and downstream signaling pathways. The KRAS gene can harbor oncogenic mutations that result in a constitutively activated protein, independent of EGFR ligand binding, rendering antibodies to the upstream EGFR ineffective. Approximately 40% of colorectal cancers have KRAS variants in codons 12 and 13 in exon 2. Another proto-oncogene that acts downstream from KRAS/NRAS harbors

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<td>Comparators of interest are:</td>
<td>Relevant outcomes include:</td>
</tr>
<tr>
<td>• With metastatic</td>
<td>• KRAS, NRAS, and BRAF variant testing</td>
<td>• Using tissue biopsy to guide treatment</td>
<td>• Overall survival</td>
</tr>
<tr>
<td>colorectal cancer</td>
<td>Using circulating tumor cell testing (liquid biopsy) to guide treatment</td>
<td></td>
<td>• Disease-specific survival</td>
</tr>
<tr>
<td></td>
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<td></td>
<td>• Change in disease status</td>
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oncogenic variants in codons 12, 13, or 61 that result in constitutive activation of the EGFR-mediated pathway. These variants are relatively rare compared with KRAS, detected in perhaps 2% to 7% of CRC specimens. It is unclear whether NRAS mutations predict poor response to anti-EGFR monoclonal antibody therapy or are prognostic of poor CRC outcome in general. A third proto-oncogene, BRAF, encodes a protein kinase and is involved in intracellular signaling and cell growth and is a principal downstream effector of KRAS. BRAF mutations occur in less than 10% to 15% of CRCs and appear to be a marker of poor prognosis. KRAS and BRAF mutations are considered to be mutually exclusive.

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

Detecting ctDNA and Circulating Tumor Cells

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

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

Highly sensitive and specific methods have been developed to detect ctDNA, for both single nucleotide variants (eg BEAMing [which combines emulsion polymerase chain reaction with magnetic beads and flow cytometry] and digital polymerase chain reaction) and copy-number variants. Digital genomic technologies allow for enumeration of rare variants in complex mixtures of DNA.

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

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

A number of liquid biopsy tests related to targeted treatment of metastatic colorectal cancer have been developed (Table 1).
**Table 1. Examples of Liquid Biopsy Tests Related to Targeted Treatment of Metastatic Colorectal Cancer**

<table>
<thead>
<tr>
<th>Manufacturer</th>
<th>Test</th>
<th>Type of Liquid Biopsy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biocept</td>
<td>Target SElector ctDNA EGFR Kit</td>
<td>ctDNA</td>
</tr>
<tr>
<td>CellMax Life</td>
<td>CellMax-CRC Colorectal Cancer Early Detection Test</td>
<td>CTC</td>
</tr>
<tr>
<td>Cynvenio</td>
<td>ClearID Solid Tumor Panel</td>
<td>ctDNA and CTC</td>
</tr>
<tr>
<td>Foundation Medicine</td>
<td>FoundationOne Liquid (Previously FoundationAct)</td>
<td>ctDNA</td>
</tr>
<tr>
<td>Guardant Health</td>
<td>Guardant360®</td>
<td>ctD</td>
</tr>
<tr>
<td>IV Diagnostics</td>
<td>Velox™</td>
<td>CTC</td>
</tr>
<tr>
<td>Pathway Genomics</td>
<td>CancerIntercept® Detect</td>
<td>ctD</td>
</tr>
<tr>
<td>Personal Genome Diagnostics</td>
<td>PlasmaSELECT</td>
<td>ctD</td>
</tr>
<tr>
<td>Sysmex Inostics</td>
<td>OncoBEAM</td>
<td>ctD</td>
</tr>
<tr>
<td>Circulogene</td>
<td>Theranostics</td>
<td>ctD</td>
</tr>
</tbody>
</table>

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

**REGULATORY STATUS**

Approved Companion Diagnostic Tests for KRAS Variant Analysis to Select Cetuximab and Panitumumab in Metastatic Colorectal Cancer

Companion diagnostic tests for the selection of cetuximab and panitumumab have been approved by FDA through the premarket approval process (Table 2):

**Table 2. Companion Diagnostic Tests for the Selection of Cetuximab and Panitumumab for Metastatic Colorectal Cancer**

<table>
<thead>
<tr>
<th>Diagnostic Name.</th>
<th>PMA/S510(k)/HDE</th>
<th>Description</th>
<th>Approval Date</th>
<th>Diagnostic Manufacturer</th>
</tr>
</thead>
<tbody>
<tr>
<td>FoundationOne CDx</td>
<td>P170019</td>
<td>Next Generation Sequencing Oncology Panel, Somatic Or Germline Variant Detection System</td>
<td>11/30/2017</td>
<td>Foundation Medicine, Inc.</td>
</tr>
<tr>
<td>Praxis</td>
<td>P160038</td>
<td>Next Generation Sequencing Oncology Panel, Somatic Or Germline Variant Detection System</td>
<td>06/29/2017</td>
<td>Illumina, Inc.</td>
</tr>
<tr>
<td>cobas KRAS Mutation Test</td>
<td>P140023</td>
<td>Somatic Gene Mutation Detection System</td>
<td></td>
<td>Roche Molecular Systems, Inc</td>
</tr>
<tr>
<td>therascreen KRAS RGQ PCR Kit</td>
<td>P110030/P110027</td>
<td>Somatic Gene Mutation Detection System</td>
<td>5/23/2014</td>
<td>Qiagen Manchester, Ltd.</td>
</tr>
</tbody>
</table>

Source: U.S. Food and Drug Administration (2019)

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

Clinical laboratories may develop and validate tests in-house and market them as a laboratory service; laboratory-developed tests must meet the general regulatory standards of the Clinical Laboratory Improvement Amendments. KRAS, NRAS, and BRAF variant analyses using polymerase chain reaction methodology are available under the auspices of the Clinical Laboratory Improvement Amendments. Laboratories that offer laboratory-developed tests must be licensed under the Clinical Laboratory Improvement Amendments for high-complexity testing. To date, FDA has chosen not to require any regulatory review of this test.
POLICY
A. KRAS variant analysis may be considered medically necessary for patients with metastatic colorectal cancer to predict nonresponse prior to planned therapy with anti-epidermal growth factor receptor (EGFR) monoclonal antibodies cetuximab or panitumumab.

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

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

D. KRAS, NRAS, and BRAF variant analysis using circulating tumor DNA or circulating tumor cell testing (liquid biopsy) to guide treatment for patients with metastatic colorectal cancer is considered experimental / investigational.

Policy Guidelines
1. 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.

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

3. 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 policy updates starting in 2017 (see Table PG1). HGVS nomenclature is recommended by the Human Genome Organization, the Human Variome Project, and by the Human Genome Variation Society itself.

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>
<tr>
<td>Familial variant</td>
<td>Disease-associated variant</td>
<td>Disease-associated variant identified in a proband for use in subsequent targeted genetic testing in first-degree relatives</td>
</tr>
</tbody>
</table>

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.

RATIONALE
The most recent literature review was performed for the period through May 29, 2019.

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

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

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

**Clinical Context and Test Purpose**
The purpose of KRAS variant testing in individuals with metastatic CRC is to determine KRAS variant status to guide treatment decisions with epidermal growth factor receptor (EGFR)-targeted therapy with the monoclonal antibodies cetuximab and panitumumab.

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

The following PICOs elements were used to select literature to inform this review.

**Patients**
The relevant population of interest includes individuals with metastatic CRC.

**Interventions**
The test being considered is KRAS variant testing.

Patients with metastatic CRC are actively managed by oncologists.

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

**Outcomes**
The beneficial outcomes of interest include progression-free survival (PFS) and overall survival (OS).

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

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

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

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

**Randomized Controlled Trials**
RCTs have performed nonconcurrent subgroup analyses of the efficacy of EGFR inhibitors in patients with wild-type vs mutated KRAS in metastatic CRC. Data from these trials have consistently shown a lack of clinical response to cetuximab and panitumumab in patients with mutated KRAS, with tumor response and prolongation of PFS observed only in wild-type KRAS patients.
Amado et al (2008) performed a subgroup analysis of KRAS tumor variants in a patient population that had previously been randomized to panitumumab or to best supportive care as third-line therapy for chemotherapy-refractory metastatic CRC (Table 3). The original study reported by Van Cutsem et al (2007), designed as a multicenter RCT, was not blinded because of expected skin toxicity related to panitumumab administration. Patients were randomized 1:1 to panitumumab or to best supportive care. Random assignment was stratified by Eastern Cooperative Oncology Group (ECOG) Performance Status (0 or 1 vs 2) and geographic region. Crossover from best supportive care to the panitumumab arm was allowed in patients who experienced disease progression. Of the 232 patients originally assigned to best supportive care alone, 176 crossed over to the panitumumab arm, at a median time to a crossover of 7 weeks (range, 6.6-7.3 weeks).

Of the 463 patients in the original trial, 427 (92%) were included in the KRAS subgroup variant analysis. A central laboratory performed the KRAS variant analysis in a blinded fashion, using formalin-fixed, paraffin-embedded tumor sections and a validated KRAS variant kit (DxS) that identifies 7 somatic variants located in codons 12 and 13 using real-time polymerase chain reaction. KRAS variant status could not be determined in 36 patients because tumor samples were not available or DNA was of insufficient or 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 four to eight 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 3, the relative effect of panitumumab on PFS was significantly greater among patients with wild-type KRAS than patients with mutated KRAS in whom no benefit from panitumumab was observed. No responders to panitumumab were identified in the mutated group, indicating a 100% positive predictive value for nonresponse in that group.

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

<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>P (n=124)</td>
<td>BSC (n=119)</td>
<td>P (n=84)</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>Median progression-free survival, wk</td>
<td>12.3</td>
<td>7.3</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 on the effect of KRAS variant status on PFS and tumor response rate endpoints are limited. However, of the 168 best supportive care patients who crossed over to panitumumab after disease progression (119 with wild-type KRAS, 77 with mutated KRAS), PFS was significantly longer among patients with wild-type KRAS (median PFS: 16.4 weeks for wild-type 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-florouracil, and irinotecan (FOLFIRI) or to FOLFIRI alone for first-line treatment, a subgroup analysis of response rate and PFS by KRAS variant status was performed by Van Cutsem et al (2009). The original trial design consisted of a central stratified permuted block randomization procedure with geographic regions and ECOG Performance Status as randomization strata. Two interim assessments of safety data were conducted by an independent data safety monitoring board.

Of the original 1198 patients, 540 had KRAS-evaluable, archival material. KRAS testing was performed using genomic DNA isolated from archived formalin-fixed, paraffin-embedded tissue, using quantitative polymerase chain reaction to detect the KRAS variant status of codons 12 and 13. It was not stated whether the KRAS variant analysis was performed blinded. KRAS variants were present in 192 (35.6%) patients. No differences were found in patient demographics or baseline characteristics between the mutated and wild-type populations, including age, sex, ECOG Performance Status, involved disease sites, and liver-limited disease. PFS and tumor response rate were assessed by a blinded, independent review committee using computed tomography scans every eight weeks. A multivariate analysis performed for PFS by patient characteristics showed a trend for PFS favoring the cetuximab plus FOLFIRI combination. The patients with wild-type KRAS who received cetuximab plus FOLFIRI showed a statistically significant improvement in median PFS and tumor response rate, whereas the mutated KRAS population did not, as summarized in Table 4.

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

<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>n</td>
<td>C+F</td>
<td>F</td>
<td>C+F</td>
</tr>
<tr>
<td>599</td>
<td>172</td>
<td>176</td>
<td>105</td>
</tr>
<tr>
<td>RR (95% CI), %</td>
<td>(42.9 to 51.0)</td>
<td>(34.8 to 42.8)</td>
<td>(51.6 to 66.7)</td>
</tr>
<tr>
<td>46.9</td>
<td>38.7</td>
<td>59.3</td>
<td>43.2</td>
</tr>
<tr>
<td>Median PFS, mo&lt;sup&gt;c&lt;/sup&gt;</td>
<td>8.9</td>
<td>8.0</td>
<td>9.9</td>
</tr>
</tbody>
</table>

Hazard ratio 0.68 (p=0.017) 1.07 (p=0.47) Adapted from Van Cutsem et al (2009).<sup>6</sup><sup>a</sup> ITT in the original CRYSTAL trial assessing C+F vs F alone as first-line therapy for metastatic colorectal cancers. <sup>b</sup> 540 patients had available archival pathology material for the KRAS variant subset analysis. <sup>c</sup> Confidence intervals for median PFS were not provided in the presentation slides.

In a third trial, the phase 2 OPUS trial, the intention-to-treat (ITT) population consisted of 337 patients randomized to cetuximab and folinic acid (leucovorin), 5-flourouracil, and oxaliplatin (FOLFOX) or to FOLFOX alone in the first-line treatment of metastatic CRC.<sup>7</sup> A 10% higher response rate (assessed by independent reviewers) was observed in the population treated with cetuximab but no difference in PFS was seen between groups. Researchers then reevaluated the efficacy in the two treatment arms based on the KRAS variant status of patients’ tumors. Of the original ITT population, 233 subjects had evaluable material for KRAS testing, and 99 (42%) were KRAS variants. The demographics or baseline characteristics were similar between the wild-type and mutated groups, including patient age, sex, ECOG Performance Status, involved disease sites, and liver-limited disease. The trial showed that the addition of cetuximab to FOLFOX
resulted in a significant improvement in response rate and PFS only in the wild-type KRAS group. Table 5 summarizes study findings.

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

<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><strong>n (KRAS evaluable)</strong></td>
<td>61 C+Fx</td>
<td>73 Fx</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>p</td>
<td>0.011</td>
<td>0.106</td>
</tr>
<tr>
<td>Odds ratio (95% CI)</td>
<td>2.54 (1.24 to 5.23)</td>
<td>0.51 (0.22 to 1.15)</td>
</tr>
<tr>
<td>Median PFS, mo&lt;sup&gt;a&lt;/sup&gt;</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).<sup>7</sup> C: cetuximab; CI: confidence interval; Fx: FOLFOX (folinic acid, 5-florouracil, and oxaliplatin); MT: mutated; PFS: progression-free survival; RR: response rate; WT: wild-type.

<sup>a</sup> Confidence intervals for median PFS were not provided in presentation slides.

In the CAIRO2 study, Tol et al (2009) analyzed tumor samples from 528 of 755 previously untreated patients with metastatic CRC who were randomized to capecitabine, oxaliplatin, and bevacizumab (CB regimen, n=378), or to the same CB regimen plus cetuximab (n=377).<sup>8</sup> KRAS variant was found in 40% of tumors (108 from patients in the CB group, 98 from the CB plus cetuximab group). Patients with KRAS variants treated with cetuximab had a significantly shorter PFS (8.1 months) than the wild-type KRAS patients who received cetuximab (10.5 months; p=0.04). In addition, patients who had mutated KRAS tumors who received cetuximab had a significantly shorter PFS and OS than patients with mutated KRAS tumors who did not receive cetuximab (PFS: 8.1 months vs 12.5 months, respectively, p=0.003; OS: 17.2 months vs 24.9 months, respectively, p=0.03). For patients with wild-type tumors, no significant PFS differences were reported between groups. Overall, patients treated with cetuximab who had tumors with a mutated KRAS gene had significantly decreased PFS compared with cetuximab-treated patients with wild-type KRAS tumors or patients with mutated KRAS tumors in the CB group.

Karapetis et al (2008) analyzed tumor samples from 394 (69%) of 572 patients with CRC who were randomized to cetuximab plus best supportive care (n=287) or to best supportive care alone (n=285) for KRAS variants and assessed whether variant status was associated with survival.<sup>9</sup> The patients had advanced CRC had failed chemotherapy and had no other standard anticancer therapy available. Of the tumors evaluated (198 from the cetuximab group, 196 from the best supportive care group), 41% and 42% had a KRAS variant, respectively, and these groups reported a median OS of 9.5 months and 4.8 months, respectively (HR for death, 0.55; 95% CI, 0.41 to 0.74; p<0.001) and a median PFS of 3.7 months and 1.9 months, respectively (HR for progression to death, 0.40; 95% CI, 0.30 to 0.54; p<0.001). For patients with mutated KRAS tumors, no significant differences were reported between those treated with cetuximab and best supportive care alone with respect to OS (HR=0.98, p=0.89) or PFS (HR=0.99, p=0.96).

Douillard et al (2010) reported on the results of a multicenter, phase 3 trial in which patients with no prior chemotherapy for metastatic CRC, ECOG Performance Status of 0 to 2, and available tissue for biomarker testing were randomized 1:1 to panitumumab plus FOLFOX4 or to FOLFOX4.<sup>10</sup> The primary endpoint was PFS; OS was a secondary endpoint. Results were prospectively analyzed on an ITT basis by tumor KRAS status. KRAS results were available for 93% of the 1183 patients randomized. In the wild-type KRAS group, panitumumab plus FOLFOX4
significantly improved PFS compared with FOLFOX4 alone (median PFS, 9.6 months vs 8.0 months, respectively; HR=0.80; 95% CI, 0.66 to 0.97; p=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. In an updated analysis of CRYSTAL, Van Cutsem et al (2011) reported on longer follow-up and more patients evaluable for tumor KRAS status and considered the clinical significance of the BRAF variant tumor status in the expanded population of patients with wild-type KRAS tumors. Subsequent to the initial published analysis, which reported an OS cutoff of December 2007, and an associated overall median duration of follow-up of 29.7 months, additional tumor analysis allowed for the typing of another 523 tumors for KRAS variant status, representing an increase in the ascertainment rate from 45% of ITT population patients in the original analysis to 89% (540 to 1063) in the current analysis, with variants detected in 37% of tumors. The updated OS analysis was carried out with a new cutoff date of May 2009, giving an overall median duration of follow-up of 46 months. The addition of cetuximab to FOLFIRI in patients with wild-type KRAS disease resulted in significant improvements in OS (median, 23.5 months vs 20.0 months; HR=0.796; p=0.009), PFS (median, 9.9 months vs 8.4 months; HR=0.696; p=0.001), and response rate (57.3% vs 39.7%; odds ratio [OR], 2.069; p<0.001) compared with FOLFIRI alone. Significant interactions between KRAS status and treatment effect were noted for all key efficacy endpoints. KRAS variant status was confirmed as a powerful predictive biomarker for the efficacy of cetuximab plus FOLFIRI. BRAF V600E variants were detected in 60 (6%) of 999 tumor samples evaluable for both BRAF and KRAS. In all but a single case, BRAF variants were identified in tumors wild-type for KRAS. The impact of BRAF tumor variant status in relation to the efficacy of cetuximab plus FOLFIRI was examined in the population of patients with wild-type KRAS disease (n=625). No evidence was reported for an independent treatment interaction by tumor BRAF variant status. The trialists concluded that BRAF variant status was not predictive of treatment effects of cetuximab plus FOLFIRI but that BRAF tumor variant was a strong indicator of poor prognosis for all efficacy endpoints compared with those whose tumors were wild-type.

Peeters et al (2010) reported on the results of a phase 3 study in which 1186 patients with metastatic CRC were randomized to panitumumab plus FOLFIRI or to FORFIRI alone as a second-line treatment. The trial endpoints were PFS and OS, which were independently tested and prospectively analyzed by KRAS status. KRAS status was available for 91% of patients: 597 (55%) had wild-type KRAS tumors and 486 (45%) had mutated KRAS tumors. In the wild-type KRAS subpopulation, when panitumumab was added to chemotherapy, a significant improvement in PFS was observed (HR=0.73; 95% CI, 0.59 to 0.90; p=0.004); median PFS was 5.9 months for panitumumab plus FOLFIRI and 3.9 months for FORFIRI. A nonsignificant trend toward increased OS was observed; median OS for panitumumab plus FOLFIRI was 14.5 months while median OS for FORFIRI alone was 12.5 months (HR=0.85, 95% CI, 0.70 to 1.04;
p=0.12). Response rates improved with the addition of panitumumab to the FOLFIRI regimen. In patients with mutated KRAS, no difference was reported in efficacy. Adverse events were comparable across arms. The trialists concluded that panitumumab plus FOLFIRI significantly improved PFS and was well-tolerated as second-line treatment in patients with wild-type KRAS metastatic CRC.

Maughan et al (2011) reported on the results of a phase 3, multicenter trial 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. The analysis was by ITT and treatment allocation was not masked. A total of 1630 patients were randomized to treatment groups (815 to standard therapy, 815 to the addition of cetuximab). Tumor samples from 1316 (81%) of patients were used for somatic variant analyses; 43% had KRAS variants. In patients with wild-type KRAS tumors, OS did not differ between treatment groups (median survival, 17.9 months in the control group vs 17.0 months in the cetuximab group; HR=1.04; 95% CI, 0.87 to 1.23; p=0.67). BRAF variants were detected in 8% of patients; BRAF did not show any evidence of a benefit from the addition of cetuximab. Contrary to other trials that have studied the benefit of adding cetuximab to the regimen of wild-type KRAS patients, this trial did not show a benefit of adding cetuximab to oxaliplatin-based chemotherapy.

Systematic Reviews
Qiu et al (2010) conducted a meta-analysis of 22 studies on the predictive and prognostic value of KRAS variants in metastatic CRC patients treated with cetuximab. The overall KRAS variant rate was 38% (829/2188 patients). Meta-analytic results were consistent with previous studies on the use of cetuximab and KRAS variant status, in that patients with tumors harboring mutant-type KRAS were more likely to have a worse response, PFS, and OS when treated with cetuximab than those with wild-type KRAS.

Dahabreh et al (2011) conducted a systematic review of RCTs that assessed the use of KRAS variant testing as a predictive biomarker for treatment of advanced CRC with cetuximab and panitumumab. Reviewers concluded that, compared with patients who had wild-type KRAS, KRAS variants were consistently associated with reduced OS and PFS and increased treatment failure rates among patients with advanced CRC who are treated with anti-EGFR antibodies.

In a pooled analysis of wild-type KRAS tumors from the CRYSTAL and OPUS trials, Bokemeyer et al (2012) assessed extended survival data and enhancement in the ascertainment rate of KRAS and BRAF tumor variant status. Pooled individual patient data from each trial were analyzed for OS, PFS, and best objective response rate (ORR) in patients evaluable for KRAS and BRAF variant status. In 845 patients with wild-type KRAS tumors, adding cetuximab to chemotherapy led to significant improvements in OS (HR=0.81; p=0.006), PFS (HR=0.66; p<0.001), and ORR (OR=2.16; p<0.001). BRAF variants were detected in 70 (8.8%) of 800 evaluable tumors. No significant differences were found in outcomes between treatment groups. However, the prognosis was worse in each treatment arm for patients with BRAF tumors, and OPUS trials confirmed the consistency of the benefit obtained from all efficacy endpoints from adding cetuximab to first-line chemotherapy in patients with wild-type KRAS metastatic CRC. It further suggested that BRAF variants do not appear to be predictive biomarkers in this setting but are markers of poor prognosis.
Single-Arm Studies
In addition to the three randomized trials discussed, a number of single-arm studies have retrospectively evaluated KRAS variant status and treatment response in patients with metastatic CRC.\textsuperscript{17,18,19,20,21} Overall they have shown similar nonresponse rates to anti-EGFR monoclonal antibodies (cetuximab, panitumumab) in patients with mutated KRAS tumors. Two of these single-arm studies have also reported differences in PFS and OS.\textsuperscript{18,21}

Section Summary: Clinically Valid
Evidence for the clinical validity of KRAS variants in predicting nonresponse to anti-EGFR monoclonal antibody therapy consists of multiple systematic reviews, including a TEC Assessment, and RCTs. The evidence has demonstrated that the presence of a KRAS variant predicts nonresponse to treatment while KRAS wild-type status predicts response to anti-EGFR monoclonal antibody therapy.

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

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

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

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

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

Section Summary: Clinically Useful
Direct evidence for the clinical validity of KRAS variant testing includes RCTs. RCTs supporting Food and Drug Administration approvals for cetuximab and panitumumab have demonstrated that the presence of KRAS variants is predictive of nonresponse to anti-EGFR monoclonal antibody therapy. Documentation of KRAS wild-type status is required before patients are eligible for treatment with cetuximab or panitumumab.
NRAS Variant Testing to Guide Treatment for Metastatic CRC

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

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

The following PICOs elements were used to select literature to inform this review.

Patients
The relevant population of interest includes individuals with metastatic CRC.

Interventions
The test being considered is NRAS variant testing.

Patients with metastatic CRC are actively managed by oncologists.

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

Outcomes
The beneficial outcomes of interest include PFS and OS.

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

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

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

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

RCTs have analyzed nonconcurrent subgroups for the efficacy of EGFR inhibitors in patients with wild-type and mutated RAS genes in metastatic CRC.

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

Table 6. 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 0.91)</td>
<td>0.007</td>
<td>0.81 (0.63 to 1.03)</td>
<td>0.08</td>
</tr>
<tr>
<td>Wild-type KRAS exon 2</td>
<td>0.73 (0.59 to 0.90)</td>
<td>0.004</td>
<td>0.85 (0.70 to 1.04)</td>
<td>0.12</td>
</tr>
</tbody>
</table>

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

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

Van Cutsem et al (2015) reported on results of a prospective-retrospective extended RAS variant analysis of tumor samples from the randomized phase 3 CRYSTAL trial, which compared FOLFIRI with FOLFIRI plus cetuximab in wild-type KRAS exon 2 patients. 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 a significant benefit in median PFS (9.9 months vs 8.4 months; p<0.05) and median OS (23.5 months vs 20 months; p<0.05) with the addition of cetuximab to chemotherapy.

Douillard et al (2013) performed a prospective-retrospective analysis of RAS variants (KRAS, NRAS) in tumor samples from patients enrolled in the Panitumumab Randomized Trial in Combination with Chemotherapy for Metastatic Colorectal Cancer to Determine Efficacy RCT. A total of 108 (17%) of 641 tumor specimens that did not harbor exon 2 KRAS variants had variants in other RAS exons, including NRAS (exons 2 or 4) and KRAS (exons 3 and 4). For patients with a wild-type KRAS exon 2 variant (n=656), OS was significantly better with panitumumab plus FOLFOX4 (n=325; median, 23.8 months) than with FOLFOX4 alone (n=331; median, 19.4 months; p=0.03). For patients with no KRAS exon 2 variant but with 1 type of RAS variant, median OS with panitumumab plus FOLFOX4 was shorter (n=51; median, 17.1 months) than with FOLFOX4 alone (n=57; median, 17.8 months; p=0.01). These data would suggest variants in a RAS gene exon other than KRAS exon 2 negatively affect anti-EGFR therapy. However, the investigators did not discriminate between specific types of RAS variants,
so it is not possible to relate NRAS to these results. Furthermore, the numbers of patients involved were very small, further limiting conclusions.

Tumor specimens (288 of 320) from an RCT by Van Cutsem et al (2007) were analyzed by Peeters et al (2013) using next-generation sequencing to investigate whether EGFR pathway variants would predict response to monotherapy with panitumumab compared with best supportive care. This 2013 analysis showed that NRAS had mutated in 14 (5%) of 282 samples with available data. Among patients with wild-type KRAS (codons 12, 13, and 61) and wild-type NRAS (n=138), treatment with panitumumab was associated with improved PFS (HR=0.39; 95% CI, 0.27 to 0.56; p<0.001) compared with best supportive care. Among those with wild-type KRAS but mutated NRAS (n=11), treatment with panitumumab was no longer associated with longer PFS (HR=1.94; 95% CI, 0.44 to 8.44; p=0.379). A treatment interaction analysis was suggestive but not significantly indicative of an interaction between the presence of mutated NRAS and poorer outcome (p=0.076). The authors suggested their data were consistent with the hypothesis that NRAS variants may limit the efficacy of anti-EGFR therapy. However, because the prevalence of NRAS variants was low, the degree of predictive or prognostic value is more uncertain.

Retrospective Cohort Studies
A retrospective consortium analysis by De Roock et al (2010) reported on results of centrally performed high-throughput mass spectrometric variant profiling of CRC specimens gathered from 11 centers in 7 European countries. Patients had been treated with panitumumab alone, cetuximab alone, or cetuximab plus chemotherapy. Among 747 of 773 samples with data, KRAS had mutated in 299 (40%), including codons 12, 13, 61, and 146. By contrast, NRAS variants were identified in 17 (2.6%) of 644 samples with data, primarily in codon 61. KRAS and NRAS variants were mutually exclusive. Among wild-type KRAS samples from patients treated with cetuximab plus chemotherapy, the NRAS variant was associated with an ORR of 7.7% (1/13) compared with 38% for the wild-type NRAS (p=0.013). However, there were no significant differences between NRAS mutant and wild-type genes in median PFS (14 weeks vs 26 weeks, p=0.055) or OS (38 weeks vs 50 weeks, p=0.051). Similar to results previously reported, the results of this analysis showed a very low prevalence of NRAS variants and were inconclusive as to whether NRAS variants are predictive of nonresponse to anti-EGFR therapy or are prognostic indicators of poor outcomes of CRC.

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

Section Summary: Clinically Valid
Evidence for the clinical validity of NRAS variants in predicting nonresponse to anti-EGFR monoclonal antibody therapy includes prospective-retrospective analyses of RCTs. Subgroup analyses of KRAS wild-type patients who did not respond to anti-EGFR monoclonal antibody therapy have suggested that NRAS variants are predictive of nonresponse. However, because of the low prevalence of NRAS variants, the predictive value of NRAS variants is uncertain.
Clinically Useful
A test is clinically useful if the use of the results informs management decisions that improve the net health outcome of care. The net health outcome can be improved if patients receive correct therapy, or more effective therapy, or avoid unnecessary therapy, or avoid unnecessary testing.

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

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

Chain of Evidence
Indirect evidence on clinical utility rests on clinical validity. If the evidence is insufficient to demonstrate test performance, no inferences can be made about clinical utility. Documentation of KRAS wild-type status is required prior to treatment with cetuximab or panitumumab.

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

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

BRAF Variant Testing to Guide Treatment for Metastatic CRC
Clinical Context and Test Purpose
The purpose of BRAF variant testing in individuals with metastatic CRC is to determine BRAF variant status to guide treatment.

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

The following PICO(s) elements were used to select literature to inform this review.

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

Interventions
The test being considered is BRAF variant testing.
Patients with metastatic CRC are actively managed by oncologists.

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

**Outcomes**
The beneficial outcomes of interest include PFS and OS.

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

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

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

**Systematic Reviews**
A meta-analysis by Pietrantonio et al (2015) identified 9, phase 3 trials that compared cetuximab or panitumumab with standard therapy or best supportive care. The analysis included 463 patients with metastatic CRC and BRAF variants. The addition of an EGFR inhibitor did not improve PFS (HR=0.88; 95% CI, 0.67 to 1.14; p=0.33) or ORR (RR=1.31; 95% CI, 0.83 to 2.08; p=0.25) compared with the control arms.

A meta-analysis by Mao et al (2011) assessed BRAF variants and resistance to anti-EGFR monoclonal antibodies in patients with metastatic CRC. The primary endpoint of eligible studies was ORR, defined as the sum of complete and partial tumor response. Eleven studies reported sample sizes ranging from 31 to 259 patients. Anti-EGFR therapy was given as first-line treatment in one study and as second-line or greater in the other ten. In two studies, the anti-EGFR monoclonal antibody was given as monotherapy, and in nine studies, patients received various chemotherapies. Seven studies were performed in unselected patients (ie, unknown KRAS variant status) totaling 546 patients, for whom 520 were assessable for tumor response. In the unselected population, a BRAF variant was detected in 8.8% of patients, and the ORR for patients with mutant BRAF was 29.2% (14/48) and for wild-type BRAF was 33.5% (158/472; p=0.048). Four studies were performed in patients with wild-type KRAS metastatic CRC. BRAF variant status was performed on 376 wild-type KRAS tumors. BRAF variant was detected in 10.6% (n=40) of primary tumors. Among the 376 analyzed, all patients were assessable for tumor response. The ORR of patients with a mutant BRAF gene was 0% (0/40), whereas the ORR of patients with wild-type BRAF was 36.3% (122/336). Only three studies presented data on PFS and OS and, therefore, a pooled analysis was not performed. Reviewers concluded that, although the meta-analysis provided evidence that BRAF variants were associated with lack of response to anti-EGFR monoclonal antibodies in wild-type KRAS metastatic CRC, the number of studies and number of patients analyzed were
relatively small and that large studies would be needed to confirm the meta-analytic results using homogenous metastatic CRC patients with assessors blinded to the clinical data.

Mao et al (2011) meta-analysis also assessed \textit{BRAF}V600E variant and resistance to anti-EGFR monoclonal antibodies in patients with metastatic CRC.\textsuperscript{30} The same 11 studies were selected. Seven included unselected patients, and four studies included only patients with wild-type K\textit{RAS}. The primary endpoint 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 K\textit{RAS}, \textit{BRAF} variant status was performed successfully on 376 wild-type K\textit{RAS} 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 K\textit{RAS} metastatic CRC treated with anti-EGFR monoclonal antibodies.

**Retrospective Studies**

Di Nicolantonio et al (2008) retrospectively analyzed 113 patients with metastatic CRC who had received cetuximab or panitumumab.\textsuperscript{32} 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{35} 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, Tol et al (2009) retrospective analyzed \textit{BRAF} variants in 516 available tumors from patients previously randomized to the CB regimen or to the CB plus cetuximab regimen.\textsuperscript{40} 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, unlike K\textit{RAS} 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{6} Analysis of \textit{BRAF} variants in this patient population and the influence of \textit{BRAF} variant status by Peeters et al (2014) showed that for the wild-type, K\textit{RAS}- and \textit{BRAF}-mutated patients, OS for cetuximab plus FOLFIRI was 14.1 months and 10.3 months with FOLFIRI (p=0.744).\textsuperscript{41} Although this difference was not statistically significant, it suggested a trend toward improved OS, PFS, and response, and that wild-type K\textit{RAS}- 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{25} Tumor samples were from fresh frozen or formalin-fixed, paraffin-embedded tissue, and the variant status was compared with retrospectively collected clinical outcomes including ORR, PFS, and OS. \textit{BRAF} variants were found in 36 (4.7\%) of 761 tumors. In patients with wild-type K\textit{RAS}, 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).
In an updated analysis of the CRYSTAL trial, Van Cutsem et al (2011) reported on longer follow-up and more patients with evaluable for KRAS tumor status and considered the clinical significance of BRAF tumor variant status in the expanded population of patients with wild-type KRAS tumors. 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 endpoints compared with those whose tumors were wild-type.

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

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

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

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

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

A chain of evidence, based on clinical validity, cannot be constructed to support the use of the anti-EGFR monoclonal antibodies cetuximab and panitumumab for the treatment of patients with wild-type BRAF metastatic CRC.

Documentation of KRAS wild-type status is required prior to treatment with cetuximab or panitumumab. Documentation of BRAF variant status is not required but has been suggested to identify patients who are predicted to be nonresponders to anti-EGFR monoclonal antibody therapy.

Section Summary: Clinically Useful
Direct evidence for the clinical validity of BRAF variant testing includes meta-analyses of prospective and retrospective analyses of RCTs. BRAF variant testing has potential clinical utility in predicting nonresponse to anti-EGFR monoclonal antibody therapy in patients with
documented \textit{KRAS} wild-type and \textit{NRAS} wild-type status. However, the direct evidence is limited for \textit{BRAF} variant testing due to the low prevalence \textit{BRAF} variants in CRC.

\textbf{Circulating Tumor DNA Testing (Liquid Biopsy) to Guide Treatment for Metastatic CRC}

\textbf{Clinical Context and Test Purpose}

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

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

The following PICOs elements were used to select literature to inform this review.

\textbf{Patients}

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

Patients with metastatic CRC are actively managed by oncologists.

\textbf{Interventions}

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

\textbf{Comparators}

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

\textbf{Outcomes}

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

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

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

\textbf{Technically Reliable}

Assessment of technical reliability focuses on specific tests and operators and requires a review of unpublished and often proprietary information. Review of specific tests, operators, and unpublished data are outside the scope of this evidence review and alternative sources exist. This evidence review focuses on the clinical validity and clinical utility.
Clinically Valid
A test must detect the presence or absence of a condition, the risk of developing a condition in the future, or treatment response (beneficial or adverse).

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

OncoBEAM RAS CRC Assay
The clinical validity of the OncoBEAM RAS CRC assay has been evaluated in several published studies of patients with metastatic CRC. Study characteristics and results are shown in Tables 7 and 8. Study relevance, design, and conduct limitations are described in Tables 11 and 12.

| Table 7. Clinical Validity Studies of the OncoBEAM RAS Assay |
|------------------|------------------|------------------|------------------|------------------|
| **Study**        | **Study Population**                                                                 | **Design**                         | **Reference Standard**                                                                 | **Timing of Tissue Biopsy and Liquid Biopsy**                                                                 | **Blinding of Assessors** |
| Garcia-Foncillas et al (2018)42. | - Patients with metastatic CRC newly diagnosed or presenting with recurrent disease after resection and/or chemotherapy at 10 centers in Spain  
- Enrolled from November 2015 to October 2016 | Prospective Analysis of tissue using standard-of-care procedures validated by each hospital | Plasma collected before any therapeutic intervention. OncoBEAM used when standard of care RAS result was discordant with RAS result. The same tissue block was used for re-analysis by OncoBEAM | Not stated; central laboratory used |
| Vidal et al (2017)43. | - Patients from Spain with histologically confirmed metastatic CRC  
- Anti-EGFR treatment-naive  
- Enrolled from 2009 to 2016 | Retrospective-prospective Analysis of tissue samples conducted using institutional standard-of-care procedures | - Tissue collected before blood  
- Median interval, 48 d (range, 0-1783 d) | Yes |
| Schmiegel (2017)44. | - Patients from Australia and Germany with newly diagnosed stage III/IV histologically confirmed CRC | Prospective Analysis of tissue samples conducted using Sanger sequencing | - Blood obtained immediately prior to tissue biopsy or resection | Not stated |
| Grasselli (2017)45. | - Patients from Spain with histologically confirmed metastatic CRC  
- Anti-EGFR treatment-naive but majority | Retrospective-prospective Analysis of tissue samples conducted using real-time PCR | Tissue collected before blood  
- Median interval 1.2 m (range 0 to 34) | Yes |
<table>
<thead>
<tr>
<th>Study</th>
<th>Study Population</th>
<th>Design</th>
<th>Reference Standard</th>
<th>Timing of Tissue Biopsy and Liquid Biopsy</th>
<th>Blinding of Assessors</th>
</tr>
</thead>
</table>
| Normanno (2018)⁴⁶,          | treated with other systemic therapies Patients with metastatic CRC who KRAS exon-2 wild-type and received first-line etuximab plus FOLFIRI within the CAPRI-GOIM trial | Retrospective-prospective | Analysis of tissue samples conducted using NGS | • Unclear when tissue was collected  
• Blood collected at baseline | Not stated |

CRC: colorectal cancer; EGFR: epidermal growth factor receptor; NGS: next-generation sequencing; PCR: polymerase chain reaction.

**Table 8. Clinical Validity Studies of the OncoBEAM RAS Assay—Results**

<table>
<thead>
<tr>
<th>Study</th>
<th>Initial N</th>
<th>Final N</th>
<th>Excluded Samples</th>
<th>RAS Variant-Positive, %ᵃ</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Garcia-Foncillas et al (2018)⁴²</td>
<td>239</td>
<td>236</td>
<td>3 patients initially excluded because of total disease removal during primary surgery. RAS mutation status was evaluable in all 236 patients</td>
<td>55.5</td>
<td>86.3</td>
<td>92.4</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>Vidal et al (2017)⁴³</td>
<td>NA</td>
<td>115</td>
<td>No description of samples excluded from comparison to tissue results</td>
<td>51</td>
<td>96 (87 to 100)ᵇ</td>
<td>90 (79 to 96)ᵇ</td>
<td>90 (79 to 96)ᵇ</td>
<td>96 (88 to 100)ᵇ</td>
</tr>
<tr>
<td>Schmiegel (2017)⁴⁴</td>
<td>102</td>
<td>98</td>
<td>N=3 (inadequate plasma DNA) N=1 (RAS mutation not confirmed in tissue when re-evaluated)</td>
<td>53</td>
<td>90 (79 to 96)</td>
<td>94 (82 to 98)</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>Grasselli (2017)⁴⁵</td>
<td>157</td>
<td>146</td>
<td>N=11 (pre-analytical requirements or lack of tumor tissue availability)</td>
<td>59</td>
<td>89 (77 to 96)ᵇ</td>
<td>90 (82 to 95)ᵇ</td>
<td>84 (74 to 91)ᵇ</td>
<td>93 (87 to 97)ᵇ</td>
</tr>
<tr>
<td>Normanno (2018)⁴⁶,</td>
<td>340</td>
<td>92</td>
<td>Tissue and plasma unavailable (not clear if tissue samples were sampled from those available or if all available were used)</td>
<td>36</td>
<td>70 (51 to 84)ᵇ</td>
<td>83 (71 to 92)ᵇ</td>
<td>70 (56 to 81)ᵇ</td>
<td>83 (74 to 89)ᵇ</td>
</tr>
</tbody>
</table>

RC: colorectal cancer; NA: not available; NPV: negative predictive value; PPV: positive predictive value.

ᵃ With tissue biopsy reference standard.

ᵇ Values are percent with 95% confidence interval.

ᵇConfidence intervals not reported in publication; calculated from data provided.
FoundationACT ctDNA Assay

The FoundationACT ctDNA assay, the predecessor of FoundationOne Liquid, was compared to tissue biopsy using the FoundationOne assay in one manufacturer-sponsored study. (Li et al 2019)\textsuperscript{47}. Study characteristics are shown in Table 9. The researchers reported results on the subset of 51 patients with KRAS, NRAS, and BRAF variants. These results are shown in Table 10. Positive percent agreement was 80% for all time points for short variants and increased to 90% for cases in which tissue and liquid biopsy were measured less than 270 days apart. Limitations of this study are described in Tables 11 and 12.

Table 9. Clinical Validity Study of the FoundationACT ctDNA Assay

<table>
<thead>
<tr>
<th>Study</th>
<th>Study Population</th>
<th>Design</th>
<th>Reference Standard</th>
<th>Timing of Reference and Index Tests</th>
<th>Blinding of Assessors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Li et al (2019)\textsuperscript{47.}</td>
<td>Patients with CRC, 74% stage IV, 19% stage III, 7% stage II</td>
<td>Prospective and retrospective</td>
<td>Previously-collected tissue biopsy with FoundationOne assay</td>
<td>Liquid biopsy testing was done at the discretion of the clinician at variable time intervals after tissue sample collection (0–709 days).</td>
<td>Not stated</td>
</tr>
</tbody>
</table>

ctDNA: circulating tumor DNA; CRC: colorectal cancer.

Table 10. Clinical Validity Study of the FoundationACT ctDNA Assay- Results

<table>
<thead>
<tr>
<th>Study</th>
<th>Initial N</th>
<th>Final N</th>
<th>Excluded Samples</th>
<th>RAS Variant- Positive, %</th>
<th>Positive Percent Agreement (95% Confidence Interval)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Li et al (2019)\textsuperscript{47.}</td>
<td>96</td>
<td>73</td>
<td>22 samples did not have detectable ctDNA</td>
<td>51/74 (92%)</td>
<td>Overall (N=73) 79% Subset with KRAS, NRAS, and BRAF variants (N=51): 80% for all timepoints 90% for cases &lt;270 days between tissue and liquid biopsy</td>
</tr>
</tbody>
</table>

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

Table 11. Relevance Limitations for Clinical Validity Studies of Liquid Biopsy in Metastatic Colorectal Cancer

<table>
<thead>
<tr>
<th>Study</th>
<th>Population\textsuperscript{a}</th>
<th>Intervention\textsuperscript{b}</th>
<th>Comparator\textsuperscript{c}</th>
<th>Outcomes\textsuperscript{d}</th>
<th>Duration of Follow-Up\textsuperscript{e}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Li et al (2019)\textsuperscript{47.}</td>
<td>4.74% had metastatic disease</td>
<td>2. Reference standard was FoundationOne assay</td>
<td>3. PPV and NPV not reported</td>
<td>2: Not clear if marketed version of test used</td>
<td>3. PPV and NPV not reported</td>
</tr>
</tbody>
</table>

Garcia-Foncillas et al (2018)\textsuperscript{42.}

Vidal et al (2017)\textsuperscript{43.}

Schmiegel (2017)\textsuperscript{44.}

---

\textsuperscript{a} Population indicates the proportion of patients with metastatic disease.

\textsuperscript{b} Intervention indicates whether the liquid biopsy was performed as a part of the standard of care or as a separate test.

\textsuperscript{c} Comparator indicates whether the liquid biopsy was compared to a tissue biopsy or another liquid biopsy.

\textsuperscript{d} Outcomes indicate the positive predictive value (PPV) and negative predictive value (NPV).

\textsuperscript{e} Duration of follow-up indicates the period of time for which the data was collected.
The study limitations stated in this table are those notable in the current review; this is not a comprehensive limitations assessment. NPV: negative predictive value; PPV: positive predictive value.

<table>
<thead>
<tr>
<th>Study</th>
<th>Population&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Intervention&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Comparator&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Outcomes&lt;sup&gt;d&lt;/sup&gt;</th>
<th>Duration of Follow-Up&lt;sup&gt;e&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grasselli (2017)&lt;sup&gt;56&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normanno (2018)&lt;sup&gt;63&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 12. Study Design and Conduct Limitations for Clinical Validity Studies of OncoBEAM RAS Assay

<table>
<thead>
<tr>
<th>Study</th>
<th>Selection&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Blinding&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Delivery of Test&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Selective Reporting&lt;sup&gt;d&lt;/sup&gt;</th>
<th>Completeness of Follow-Up&lt;sup&gt;e&lt;/sup&gt;</th>
<th>Statistical&lt;sup&gt;f&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Li et al (2019)&lt;sup&gt;47&lt;/sup&gt;</td>
<td>2. Inclusion required a previously performed FoundationACT assay; previous treatments varied</td>
<td>1: blinding unclear</td>
<td>2. timing of liquid biopsy and tissue biopsy varied (range 0-709 days)</td>
<td></td>
<td>2. 20% of samples had no detectable ctDNA</td>
<td></td>
</tr>
<tr>
<td>Garcia-Foncillas et al (2018)&lt;sup&gt;42&lt;/sup&gt;</td>
<td>1. Not clear whether samples were consecutive or convenience</td>
<td>1: blinding unclear</td>
<td>1. Registration not described</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vidal et al (2017)&lt;sup&gt;43&lt;/sup&gt;</td>
<td>1. Not clear whether samples were consecutive or convenience</td>
<td>1: blinding unclear</td>
<td>2: Blood collected approximately 1.5 m after tissue</td>
<td>1. Registration not described</td>
<td>1. Not clear whether there were samples that were insufficient for analysis or failed to produce results</td>
<td>1. CIs not reported but calculated based on data provided</td>
</tr>
<tr>
<td>Schmiegel (2017)&lt;sup&gt;44&lt;/sup&gt;</td>
<td>1: Not clear how patients were selected from those that were eligible</td>
<td>1: Blinding unclear</td>
<td>1. Registration not described</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grasselli (2017)&lt;span class=&quot;reference&quot; data-end=&quot;52&quot;&gt;[52]&lt;span class=&quot;reference&quot; data-end=&quot;52&quot;&gt;&lt;/span&gt;</td>
<td>1: Not clear how patients were selected from those that were eligible</td>
<td>2: Blood collected approximately 1.5 m after tissue</td>
<td>1. Registration not described</td>
<td></td>
<td>1. CIs not reported but calculated based on data provided</td>
<td></td>
</tr>
</tbody>
</table>
The study limitations stated in this table are those notable in the current review; this is not a comprehensive limitations assessment.

<table>
<thead>
<tr>
<th>Study</th>
<th>Selection&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Blinding&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Delivery of Test&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Selective Reporting&lt;sup&gt;d&lt;/sup&gt;</th>
<th>Completeness of Follow-Up&lt;sup&gt;e&lt;/sup&gt;</th>
<th>Statistical&lt;sup&gt;f&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normanno (2018)&lt;sup&gt;46&lt;/sup&gt;</td>
<td>1: Not clear how tumor samples were selected from those available</td>
<td>1: Blinding unclear</td>
<td>1: Unclear when tissue was collected</td>
<td>1. Registration not described</td>
<td>2: Only 27% of CAPRI-GOIM trial participants included</td>
<td>1. CIs not reported but calculated based on data provided</td>
</tr>
</tbody>
</table>

<sup>a</sup> Selection key: 1. Selection not described; 2. Selection not random or consecutive (ie, convenience).

<sup>b</sup> Blinding key: 1. Not blinded to results of reference or other comparator tests.

<sup>c</sup> Test Delivery key: 1. Timing of delivery of index or reference test not described; 2. Timing of index and comparator tests not same; 3. Procedure for interpreting tests not described; 4. Expertise of evaluators not described.

<sup>d</sup> Selective Reporting key: 1. Not registered; 2. Evidence of selective reporting; 3. Evidence of selective publication.

<sup>e</sup> Follow-Up key: 1. Inadequate description of indeterminate and missing samples; 2. High number of samples/patients excluded; 3. High loss to follow-up or missing data.

<sup>f</sup> Statistical key: 1. Confidence intervals and/or p values not reported; 2. Comparison to other tests not reported.

**Clinically Useful**

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

**Direct Evidence**

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

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

**Chain of Evidence**

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

**Section Summary: Clinically Useful**

The clinical validity of the OncoBEAM RAS CRC Assay has been studied in multiple observational studies. When compared to tissue biopsy, sensitivity ranged from 70% (51% to 84%) to 96% (95% CI 87% to 100%) and specificity ranged from 83% (95% CI 71% to 92%) to 94% (82% to 98%). FoundationOne Liquid has been compared to tissue biopsy with the FoundationACT assay in one observational study; positive percent agreement was 80% overall and 90% when tissue and liquid biopsy were collected less than 270 days apart. Clinical validity studies were limited by unclear reporting of blinding, use of convenience rather than consecutive samples, and variation in the timing of sample collection. There are no published studies reporting clinical outcomes or clinical utility.

**SUMMARY OF EVIDENCE**

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

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

Clinical input obtained in 2017 supports that the following indication provides a clinically meaningful improvement in net health outcome and is consistent with generally accepted medical practice.

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

Thus, the above indication may be considered medically necessary considering the suggestive evidence and clinical input support.

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

**CLINICAL INPUT FROM PHYSICIAN SPECIALTY SOCIETIES AND ACADEMIC MEDICAL CENTERS**

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

**2017 Input**

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

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

- Use of \( \text{BRAF}^\text{V600E} \) variant analysis in individuals with metastatic colorectal cancer who are found to be wild-type on \( \text{KRAS} \) and \( \text{NRAS} \) variant analysis to guide management decisions.

**PRACTICE GUIDELINES AND POSITION STATEMENTS**

**National Comprehensive Cancer Network**

The National Comprehensive Cancer Network (v.2.2018) guidelines on the treatment of colon cancer recommend that tumor tissue should be genotyped for \( \text{RAS} \) (\( \text{KRAS} \) and \( \text{NRAS} \)) and \( \text{BRAF} \) variants, individually or as part of a next-generation sequencing panel, for all patients with metastatic colon cancer (v.2.2019). Testing should be performed on archived specimens of the primary tumor or 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 \( \text{KRAS} \) and \( \text{NRAS} \) genes. Individuals with \( \text{KRAS} \) variant in exons 2, 3, or 4, or with \( \text{NRAS} \) variant in exons 2, 3, or 4, are not eligible for treatment with cetuximab or panitumumab. The guidelines also state that the presence of the \( \text{BRAF}^\text{V600E} \) variant makes a response to panitumumab and cetuximab highly unlikely. However, the concurrent administration of a \( \text{BRAF} \) inhibitor may make a response to these treatments more likely.

The guidelines for colon cancer (v.2.2019) reference a paper on circulating tumor DNA in the discussion of adjuvant chemotherapy in stage II disease with the statement "Research into additional possible predictive markers may allow for more informed decision-making in the future."

**American College of Medical Genetics and Genomics**

An evidence review published by the American College of Medical Genetics and Genomics (2013) has stated that evidence is insufficient to support the clinical validity or utility of testing colorectal cancer specimens for \( \text{NRAS} \) variants to guide patient management. That same review further found no guidelines on \( \text{NRAS} \) testing from any other U.S. group.
The American Society of Clinical Oncology along with American Society for Clinical Pathology, College of American Pathologists, Association for Molecular Pathology (2017) published guidelines on molecular biomarkers for the evaluation of colorectal cancer. Table 13 summarizes the relevant guidelines.

**Table 13. Summary of Recommendations**

<table>
<thead>
<tr>
<th>Guidelines</th>
<th>Type</th>
<th>SOE</th>
<th>QOE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colorectal carcinoma patients being considered for anti-EGFR therapy must receive RAS mutational testing. Mutational analysis should include KRAS and NRAS codons 12, 13 of exon 2; 59, 61 of exon 3; and 117 and 146 of exon 4 (&quot;expanded&quot; or &quot;extended&quot; RAS)</td>
<td>Recommendation</td>
<td>Convincing/ adequate, benefits outweigh harms</td>
<td>High/intermediate</td>
</tr>
<tr>
<td>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)</td>
<td>Recommendation</td>
<td>Adequate/inadequate, balance of benefits and harms</td>
<td>Intermediate/low</td>
</tr>
<tr>
<td>BRAF p.V600 mutational analysis should be performed in deficient MMR tumors with loss of MLH1 to evaluate for Lynch Syndrome risk. Presence of a BRAF mutation strongly favors sporadic pathogenesis. The absence of BRAF mutation does not exclude risk of Lynch syndrome</td>
<td>Recommendation</td>
<td>Adequate/inadequate, balance of benefits and harms</td>
<td>Intermediate/low</td>
</tr>
<tr>
<td>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</td>
<td>Recommendation</td>
<td>Adequate/inadequate, balance of benefits and harms</td>
<td>Intermediate/low</td>
</tr>
<tr>
<td>There is insufficient evidence to recommend BRAF c.1799 p.V600 mutational status as a predictive molecular biomarker for response to anti-EGFR inhibitors</td>
<td>No recommendation</td>
<td>Insufficient, benefits/harms balance unknown</td>
<td>Insufficient</td>
</tr>
</tbody>
</table>

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

The American Society of Clinical Oncology (2015) updated its provisional clinical opinion 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 were small, and there was inadequate evidence to provide a definitive opinion on the lack of benefit for the use of anti-epidermal growth factor receptor antibodies for patients whose cancer harbors any specific RAS variant other than the exon 2 KRAS variant. The Society considered the less common RAS variants as a group, and a pooled analysis suggested the same lack of benefit with anti-epidermal growth factor receptor therapy as seen with the more common variants in exon 2 of KRAS.

**U.S. PREVENTIVE SERVICES TASK FORCE RECOMMENDATIONS**

Not applicable.
ONGOING AND UNPUBLISHED CLINICAL TRIALS
A currently unpublished trial that might influence this review is listed in Table 14.

Table 14. Summary of Key Ongoing Trial

<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>Investigation of the Value of ctDNA Analysis in the Diagnosis, Treatment, and Surveillance of Patients With Surgically Resectable Colorectal Cancer</td>
<td>300</td>
<td>Dec 2021</td>
</tr>
</tbody>
</table>

CODING
The following codes for treatment and procedures applicable to this policy are included below for informational purposes. Inclusion or exclusion of a procedure, diagnosis or device code(s) does not constitute or imply member coverage or provider reimbursement. Please refer to the member’s contract benefits in effect at the time of service to determine coverage or non-coverage of these services as it applies to an individual member.

CPT/HCPCS

- **81210** BRAF (B-Raf proto-oncogene, serine/threonine kinase) (eg, colon cancer, melanoma), gene analysis, V600 variant(s)
- **81275** KRAS (Kirsten rat sarcoma viral oncogene homolog) (eg, carcinoma) gene analysis; variants in exon 2 (eg, codons 12 and 13)
- **81276** KRAS (Kirsten rat sarcoma viral oncogene homolog) (eg, carcinoma) gene analysis; additional variant(s) (eg, codon 61, codon 146)
- **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)
- **86152** Cell enumeration using immunologic selection and identification in fluid specimen (eg, circulating tumor cells in blood);
- **86153** Cell enumeration using immunologic selection and identification in fluid specimen (eg, circulating tumor cells in blood); physician interpretation and report, when required
- **88363** Examination and selection of retrieved archival (ie, previously diagnosed) tissue(s) for molecular analysis (eg, KRAS mutational analysis)
- **0069U** Oncology (colorectal), microRNA, RT-PCR expression profiling of miR-31-3p, formalin-fixed paraffin-embedded tissue, algorithm reported as an expression score
- **0111U** Oncology (colon cancer), targeted KRAS (codons 12, 13 and 61) and NRAS (codons 12, 13 and 61) gene analysis utilizing formalin-fixed paraffin-embedded tissue

ICD-10 Diagnoses

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

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

09-27-2019
Updated Description section.
In Policy section:
• Added new Item D, “KRAS, NRAS, and BRAF variant analysis using circulating tumor DNA or circulating tumor cell testing (liquid biopsy) to guide treatment for patients with metastatic colorectal cancer is considered experimental / investigational.”
Updated Rationale section.
In Coding section:
• Added CPT codes: 86152, 86153, 0069U.
• Removed coding bullets.
Updated References section.

10-01-2019
In Coding section:
• Added PLA code: 0111U

REFERENCES


