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Panitumumab Therapy and *RAS* and *BRAF* Genotype

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Introduction

Panitumumab (brand name Vectibix) is a monoclonal antibody used for the treatment of metastatic colorectal cancer (mCRC). Panitumumab is an epidermal growth factor receptor (EGFR) antagonist, which works by blocking the growth of cancer cells. It is administered every 14 days as an intravenous (IV) infusion, often with chemotherapy. Panitumumab is approved for first-line therapy with folinic acid, fluorouracil, and oxaliplatin (FOLFOX) and as monotherapy following disease progression after prior treatment with fluoropyrimidine-, oxaliplatin-, and irinotecan-containing chemotherapy (1).

The location of the primary tumor correlates whether an individual with mCRC is likely respond to anti-EGFR therapy. Individuals with left-sided tumors are more likely to respond well to anti-EGFR therapy and have a better prognosis. Individuals with right-sided tumors have a worse prognosis and respond poorly to anti-EGFR therapy. However, only the genetic variation status of the tumor, and not the location of the tumor, is discussed in the FDA drug label's dosing recommendations.

Resistance to panitumumab is associated with specific RAS mutations. The RAS is a family of oncogenes that includes the *KRAS* and *NRAS* genes. When mutated, these genes have the ability to transform normal cells into cancerous cells by providing a continual growth stimulus to cells. The *KRAS* mutations are particularly common, being detectable in 40% of metastatic colorectal tumors.

The *KRAS* mutations often lead to constitutive activation of the EGFR and are associated with resistance to anti-EGFR drugs such as panitumumab. Mutations in *NRAS* and another gene, *BRAF*, have also been associated with poor response to anti-EGFR therapy.

The 2017 FDA-approved label states that panitumumab is indicated for wild-type RAS (no mutations in either *KRAS* or *NRAS*) mCRC (Table 1). The label states that an FDA-approved test must be used to confirm the absence of RAS mutations before starting panitumumab, and that panitumumab is not indicated for the treatment of individuals with colorectal cancer with RAS mutations (in either *NRAS* or *KRAS*), or when the RAS genetic variation status is unknown (1).

Similarly, the 2015 Update from the American Society of Clinical Oncology (ASCO) states that anti-EGFR therapy should only be considered for the treatment of individuals whose tumor is determined to not have variations detected after extended RAS testing (Table 2) (2).

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The 2020 National Comprehensive Cancer Network (NCCN) guideline also strongly recommends *KRAS/NRAS* genotyping of tumor tissue in all individuals with mCRC. In addition, the guideline states the V600E mutation in the *BRAF* gene makes a response to panitumumab highly unlikely, unless given with a BRAF inhibitor (Table 3) (3).

Table 1. The FDA Drug Label for Panitumumab: Dosage and Administration (2017)

Genes to be tested	Recommendations for metastatic colorectal cancer
KRAS *NRAS*	Panitumumab is not indicated for the treatment of individuals with colorectal cancer that harbor somatic mutations in exon 2 (codons 12 and 13), exon 3 (codons 59 and 61), and exon 4 (codons 117 and 146) of either *KRAS* or *NRAS* and hereafter is referred to as "RAS". Prior to initiation of treatment with panitumumab, assess RAS mutational status in colorectal tumors and confirm the absence of a RAS mutation in exon 2 (codons 12 and 13), exon 3 (codons 59 and 61), and exon 4 (codons 117 and 146) of both *KRAS* and *NRAS*. Information on FDA-approved tests for the detection of *KRAS* mutations in individuals with metastatic colorectal cancer is available at: http://www.fda.gov/CompanionDiagnostics.

This FDA table is adapted from (1).

Table 2. The ASCO RAS Mutational Testing of Colorectal Carcinoma Tissue (2015)

Genes to be tested	Recommendation
KRAS *NRAS*	RAS mutational testing of colorectal carcinoma tissue should be performed for all individuals who are being considered for anti-EGFR monoclonal antibody therapy (cetuximab and panitumumab). Before treatment with anti-EGFR antibody therapy, individuals with mCRC should have their tumor tested for mutations in: • *KRAS* exons 2 (codons 12 and 13), 3 (codons 59 and 61) and 4 (codons 117 and 146) • *NRAS* exons 2 (codons 12 and 13), 3 (codons 59 and 61), and 4 (codons 117 and 146) Anti-EGFR antibody therapy should only be considered for treatment of individuals with metastatic colorectal carcinoma who are identified as having tumors with no mutations detected after such extended RAS mutation analysis.

This ASCO table is adapted from (2). EGFR, epidermal growth factor receptor; mCRC, metastatic colorectal cancer; ASCO, American Society of Clinical Oncology

Table 3. The NCCN *KRAS*, *NRAS*, and *BRAF* Mutation Testing (2020)

Genes to be tested	Recommendations for colorectal cancer
KRAS *NRAS*	All individuals with metastatic colorectal cancer should have tumor tissue genotyped for RAS (KRAS and NRAS) and BRAF mutations. Individuals with any known KRAS mutation (exon 2, 3, 4) or NRAS mutation (exon 2, 3, 4) should not be treated with either cetuximab or panitumumab.
BRAF	BRAF V600E mutation makes response to cetuximab or panitumumab highly unlikely unless given with a BRAF inhibitor.

This NCCN table is adapted from (3). NCCN, National Comprehensive Cancer Network

Drug: Panitumumab

Panitumumab is an EGFR antagonist and is used for the treatment of mCRC. Panitumumab, and the related drug cetuximab (brand name Erbitux), are monoclonal antibodies that specifically target the extracellular domain of EGFR. They act by blocking endogenous ligand binding to the extracellular domain of EGFR, and by enhancing receptor internalization and degradation (4).

Panitumumab is a fully human monoclonal antibody, whereas cetuximab a chimeric monoclonal antibody, being composed of regions of both murine and human antibody. Both drugs have been shown to provide a clear clinical benefit in the treatment of RAS wild-type mCRC (5, 6).

Colorectal cancer is the second leading cause of cancer death for men and women in the US, and the second in Europe (7, 8). Surgery is the most common treatment for localized colorectal cancer that has not spread. Chemotherapy alone, or with radiation, is given before (neoadjuvant) or after (adjuvant) surgery to most individuals with cancer that has penetrated the bowel wall deeply or spread to the lymph nodes (9). In the context of localized colon cancer, surgery first with adjuvant chemotherapy is standard. For localized rectal cancer, chemotherapy and chemoradiation can be used before or after surgery; standard therapy is either chemoradiation followed by surgery and adjuvant chemotherapy or chemoradiation and chemotherapy followed by surgery (10). National Comprehensive Cancer Network (NCCN) guidelines state there is a lack of definitive data or clear benefit from multiple types of adjuvant therapy for stage II or III colon cancer (3).

Treatment regimens for advanced or metastatic colorectal carcinoma include drugs such as folinic acid, fluorouracil, irinotecan, capecitabine, and oxaliplatin. Targeted biological agents may be added to such regimens, such as panitumumab, cetuximab, bevacizumab, ziv-aflibercept and ramucirumab. Bevacizumab (brand name Avastin) is a monoclonal antibody that targets vascular endothelial growth factor, VEGF. The NCCN guidelines provide further detail regarding which combinations are recommended based on tumor pathology (3).

Panitumumab is used with FOLFOX (FOLinic acid, Fluorouracil, and OXaliplatin) as a first-line treatment. It can also be combined with second-line chemotherapy (11). Additionally, it is used a single agent (monotherapy) following disease progression after chemotherapy including fluoropyrimidine, oxaliplatin, and irinotecan (with or without anti-VEGF therapy) (12, 13, 14, 15, 16). Although panitumumab is not indicated as first-line therapy in combination with irinotecan-based regimes, this may be an appropriate therapy for specific individuals (17, 18, 19). However, the NCCN advises against combining panitumumab with a bevacizumab-containing regimen due to inferior outcomes and toxicity (3, 20, 21).

Of note, the location of the primary colorectal tumor is a predictor of the prognosis for metastatic disease. Left-sided tumors derive from the embryonic hindgut (which gives rise to the splenic flexure, descending colon, sigmoid colon, rectum, and one-third of the transverse colon). Whereas right-sided tumors derive from the embryonic midgut (which gives rise to the appendix, cecum, ascending colon, hepatic flexure, and two-thirds of the transverse colon) (22). The ASCO recommends that individuals with left-sided tumors *versus* right-sided tumors are more likely to receive benefit of anti-EGFR therapy in a first-line, maximal-resourced setting (23).

Individuals with left-sided tumors benefit more from EGFR therapy than individuals with right-sided tumors. Panitumumab appears to have no meaningful activity for right-sided tumors, except perhaps where early response and tumor shrinkage is an important indicator (24). Right-sided tumors may respond to bevacizumab (25, 26, 27, 28, 29).

Administration of intravenous anti-EGFR therapy is associated with severe infusion reactions, including anaphylaxis (1% for panitumumab and 3% for cetuximab). Others include cardiopulmonary arrest, severe skin rashes (the severity of which may predict an increased response and survival (30, 31)), and an increased risk of venous thrombosis and embolism (2, 9)

Pregnant women and females of childbearing age should be advised that panitumumab can cause fetal harm. Based on data from animal studies (cynomolgus monkeys), panitumumab can be lethal to embryos. Therefore, females of reproductive potential should use effective contraception during panitumumab therapy, and for at least 2 months after the last dose of panitumumab. Individuals who know or suspect they are pregnant should inform their healthcare provider.

An important role in the progression of mCRC is thought to involve the impaired regulation of EGFR function, resulting in activation of the associated mitogen-activated protein kinase (MAPK) pathway. Panitumumab and cetuximab are important drugs in metastatic disease because they can block the activation of the MAPK pathway. However, drug resistance can arise through constitutive activation of the MAPK pathway, caused by variation in downstream signaling proteins, such as KRAS, NRAS and BRAF. Approximately 40% of cases of mCRC are found to have activating mutations in *KRAS* (32, 33).

The efficacy of panitumumab in treating mCRC is confined to individuals with wild-type *KRAS* tumors. Specifically, tumors that do not harbor specific mutations in exons 2, 3, and 4 of the *KRAS* gene. The *NRAS* gene is highly similar (homologous) to *KRAS*, and has mutations in the same exons—2, 3, and 4—which are also associated with a lack of response to panitumumab (33, 34, 35).

Therefore, expanded RAS testing (of KRAS and NRAS) is the standard of care to determine which individuals with mCRC will benefit from anti-EGFR therapy (36, 37).

Proto-oncogenes

Proto-oncogenes are a group of genes that, when mutated or expressed at abnormally high levels, can contribute to normal cells becoming cancerous cells. The mutated version of the proto-oncogene is called an oncogene.

Proto-oncogenes typically encode proteins that stimulate cell division, inhibit cell differentiation, and halt cell death. All these are important biological processes. However, the increased production of these proteins, caused by oncogenes, can lead to the proliferation of poorly differentiated cancer cells (9). Members of the RAS family and the *EGFR* gene are all proto-oncogenes.

The RAS family contains 3 genes, *HRAS*, *NRAS*, and *KRAS*, and they are essential components of signaling pathways. They act as signal transducers –– coupling cell surface receptors to intracellular signaling pathways.

The RAS proteins regulate cell signal transduction by acting as a switch –– they cycle between "on" (GTP-bound) or "off" (GDP-bound) conformations. In the "on" position, RAS proteins transmit extracellular growth signals to the nucleus, primarily by the MAPK pathway. Cells are subsequently stimulated to grow, divide, mature, and differentiate.

Variation in *RAS* genes leads to RAS proteins that are resistant to GTPase, so that GTP-remains permanently bound and the receptor remains "on" –– providing a continual growth stimulus to cells. Such activating RAS mutations are common in colorectal cancers.

Gene: *KRAS*

The *KRAS* gene is the most frequently mutated RAS gene found in metastatic colorectal carcinoma. The most frequent individual mutations occur in *KRAS* exon 2, in codons 12 (G12D, G12V) and 13 (G13D). Collectively, these mutations account for more than 60% of all RAS mutations in mCRC (38). Individuals with mCRC that harbor *KRAS* mutations do not benefit from anti–EGFR therapy (either panitumumab or cetuximab therapy) (3, 5, 33, 34, 39).

Gene: *NRAS*

The *NRAS* gene is highly homologous to *KRAS*, and mutations have been reported in exons 2, 3, and 4. Although NRAS mutations are not as frequent as *KRAS* in mCRC, occurring in approximately 2% of tumors, *NRAS* influences the response to treatment with anti-EGFR drugs (2, 40, 41, 42).

Individuals with *NRAS*-mutated tumors are less likely to respond to panitumumab or cetuximab (34, 39, 43). Furthermore, panitumumab may even have a detrimental effect in individuals with *NRAS* or *KRAS* mutations (2, 39) .

Gene: *BRAF*

The RAF proteins are a family of serine/threonine kinases that are downstream effectors of KRAS, within the MAPK signaling pathway. The RAF family has 3 members, *ARAF*, *BRAF* and *CRAF* (44).

The *BRAF* mutations are detectable in approximately 5–15% of mCRC individuals. They tend to only occur in tumors that do not have *KRAS* exon 2 mutations (45). It is therefore unlikely that tumors with *KRAS* mutations will respond to either anti-BRAF treatment (which targets mutant BRAF) or anti-EGFR treatment (because of the presence of *KRAS* mutations) (46).

By far the most common *BRAF* mutation is known as V600E, which accounts for approximately 90% of *BRAF* mutations. The resulting BRAF V600E protein is constitutively active and is a highly potent oncogene, acting downstream in the EGFR pathway, thus bypassing inhibition of EGFR by panitumumab or cetuximab (7). Constitutively active BRAF can then activate the downstream kinases MEK1 and MEK2, which ultimately activate ERK kinases at the terminus of the MAP kinase signaling pathway (47).

The *BRAF* V600E mutation is associated with a poorer diagnosis for individuals with mCRC, as well as with resistance to anti-EGFR treatment. It is also possible that other *BRAF* mutations contribute to anti-EGFR resistance. In *BRAF* V600E-mutant mCRC, BRAF inhibition results in rapid feedback activation of EGFR, a likely mechanistic explanation for limited clinical utility of this monotherapy (48). Alternative treatments may include the use of drug combinations, such as the addition of a BRAF inhibitor to anti-EGFR, to overcome resistance (36, 49). Indeed, utilization of BRAF inhibitor therapy with anti-EGFR (with or without additional targeting of MEK kinases) showed improved survival in the BEACON trial, with the greatest overall survival in the group targeting BRAF, EGFR and MEK simultaneously (48, 50). Guidelines from the NCCN recommend this triple therapy as one approach for *BRAF* V600E mutation-positive disease (3). The NCCN guidelines recommend additional combination therapies for *BRAF* V600E positive CRC of either vemurafenib, irinotecan and anti-EGFR monoclonal antibodies (cetuximab or panitumumab) or dabrafenib, trametinib and anti-EGFR monoclonal antibodies (3).

The NCCN Colon/Rectal Cancer Panel states that evidence increasingly suggests that the *BRAF* V600E variant makes response to panitumumab or cetuximab, as single agents or with cytotoxic chemotherapy, highly unlikely unless it is also given with a BRAF inhibitor. Therefore, the panel recommends *BRAF* genotyping of tumor tissue (either primary tumor or metastasis) at diagnosis of stage IV disease (3).

Gene: *EGFR*

The human epidermal growth factor receptor (HER) family consists of 4 members: the *EGFR*, *ERBB2 (HER2)*, *ERBB3 (HER3)*, and *ERBB4 (HER4)*. All 4 members are transmembrane tyrosine kinase receptors, and they regulate a number of important cellular processes, such as cell growth, survival, and differentiation.

The EGFR is expressed in many different tissues, and is activated by the binding of a ligand, such as epithelial growth factor or transforming growth factor α. Binding induces receptor dimerization, either homodimers or heterodimers with other HER family members, and triggers autophosphorylation of the intracellular tyrosine kinase domain.

By activating downstream signaling pathways, EGFR has many different biological roles, including stimulating the cell cycle, cell growth, division, differentiation, as well as increased cell invasiveness, apoptosis, and

angiogenesis. Therefore, overexpression of EGFR is thought to be an important step in tumor progression, making the EGFR a target for anticancer drugs (51, 52, 53).

There are 2 classes of drug that target EGFR: tyrosine kinase inhibitors (for example, gefitinib and erlotinib) and anti-EGFR monoclonal antibodies (for example, cetuximab and panitumumab) (4).

The EGFR is overexpressed in several cancers, including squamous cell carcinoma of the head and neck, squamous cell lung cancer, and colorectal cancer. The EGFR is overexpressed in approximately 50–80% of colorectal tumors (2, 51). However, for colorectal cancer, EGFR expression has not been associated with efficacy of anti-EGFR therapy (54).

The NCCN Colon/Rectal Cancer Panel states that EGFR testing of colorectal tumor cells has no proven predictive value in determining likelihood of response to either panitumumab or cetuximab. Therefore, the panel does not recommend routine EGFR testing, and states that no individual should be considered for or excluded from cetuximab or panitumumab therapy based on EGFR test results (3).

Gene: *HER2/ERBB2*

The HER2 receptor belongs to the same family of signaling kinase receptors as EGFR and is encoded by the gene *ERBB2*, also called *HER2*. Monoclonal antibodies that target HER2, such as pertuzumab and trastuzumab, are used in the treatment of breast cancer. However, HER2 is rarely expressed in colorectal tumors (approximately 3% overall), though the prevalence is higher in *RAS/BRAF* wild-type tumors (5–14%) (3). Initial evidence suggested that HER2 overexpression may be predictive of resistance to anti-EGFR therapy, yet some evidence suggested that HER2 status is not a biomarker for anti-EGFR response (36, 55) A recent review of HER2 retrospective studies found a consistent correlation between HER2 amplification and resistance to anti-EGFR treatment (56).

The NCCN Colon/Rectal Cancer Panel recommends *HER2* amplification/overexpression testing for individuals with mCRC. However, if the tumor is known to have a *RAS* or *BRAF* mutation, *HER2* testing is not required. Based on the outcome of *HER2* testing, the individual may be eligible for enrollment in one of the on-going clinical trials investigating targeted HER2 therapy in mCRC (3). The NCCN guidelines emphasize that *HER2* overexpression is not prognostic, but can be used to predict success of HER2-targeted therapy and resistance to anti-EGFR antibodies, including panitumumab (3).

Linking gene variation with treatment response

Specific mutations in the genes *KRAS* and *NRAS* result in resistance to panitumumab therapy. In addition, the presence of the *BRAF* V600E mutation makes a beneficial response to monotherapy treatment unlikely, combination therapy targeting BRAF and EGFR is recommended in these circumstances. Mutations in *EGFR* do not appear to be associated with panitumumab resistance. Initial results suggest *HER2* amplification may predict resistance to panitumumab (reviewed in (3)).

Genetic Testing

The NIH Genetic Testing Registry, GTR, displays genetic tests that are available for the panitumumab drug response, and the genes *KRAS*, *NRAS*, *BRAF*, *HER2,* and *EGFR*

The 2020 NCCN Guideline for Colon Cancer (Version 4.2020) provides the following recommendations for genetic testing:

KRAS, NRAS, and BRAF Mutation Testing

- All [individuals] with metastatic colorectal cancer should have tumor tissue genotyped for *RAS* (*KRAS* and *NRAS*) and *BRAF* [variants] individually or as part of an NGS panel. [Individuals] with any known *KRAS* mutation (exon 2, 3, 4) or *NRAS* mutation (exon 2, 3, 4) should not be treated with either cetuximab or panitumumab. *BRAF* V600E mutation makes response to panitumumab or cetuximab highly unlikely unless given with a BRAF inhibitor.
- No specific methodology is recommended (e.g., sequencing, hybridization) for testing *KRAS*, *NRAS*, and *BRAF* mutations.
- The testing can be performed on formalin-fixed paraffin-embedded tissue. The testing can be performed on the primary colorectal cancers and/or the metastasis, as literature has shown that the *KRAS*, *NRAS*, and *BRAF* mutations are similar in both specimen types.

Microsatellite Instability (MSI) or Mismatch Repair (MMR) Testing

- Universal MMR* or MSI* testing is recommended in all newly diagnosed [individuals] with colon cancer. See NCCN Guidelines for Genetic/Familial High-Risk Assessment: Colorectal (*IHC for MMR and DNA analysis for MSI are different assays and measure different biological effects caused by deficient MMR function)
- The presence of a *BRAF* V600E mutation in the setting of *MLH1* absence would preclude the diagnosis of Lynch syndrome (LS) in the vast majority of cases. However, approximately 1% of cancers with *BRAF* V600E mutation (and loss of MLH-1) are LS. Caution should be exercised in excluding cases with a strong family history from germline screening in the case of *BRAF* V600E mutations.
- Stage II MSI-H [individuals] may have a good prognosis […]
- Testing for MSI may be accomplished by polymerase chain reaction or a validated NGS panel, especially in [individuals] with metastatic disease who require genotyping of *RAS* and *BRAF* (3).

Therapeutic Recommendations based on Genotype

This section contains excerpted[1] information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

2017 Statement from the US Food and Drug Administration (FDA):

Prior to initiation of treatment with panitumumab, assess RAS mutational status in colorectal tumors and confirm the absence of a RAS mutation. Information on FDA-approved tests for the detection of KRAS mutations in individuals with metastatic colorectal cancer is available at: http://www.fda.gov/CompanionDiagnostics.

[…]

Panitumumab is not indicated for the treatment of individuals with colorectal cancer that harbor somatic mutations in exon 2 (codons 12 and 13), exon 3 (codons 59 and 61), and exon 4 (codons 117 and 146) of either KRAS or NRAS and hereafter is referred to as "RAS".

Please review the complete therapeutic recommendations that are located here: (1)

2015 Provisional Clinical Opinion from the American Society of Clinical Oncology (ASCO) and 2020 Late-Stage Colorectal Cancer ASCO Resource-Stratified Guidelines
All individuals with metastatic colorectal cancer who are candidates for anti-EGFR antibody therapy should

[1] The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labeled all formulations containing the generic drug.

have their tumor tested in a Clinical Laboratory Improvement Amendments–certified laboratory for mutations in both KRAS and NRAS exons 2 (codons 12 and 13), 3 (codons 59 and 61), and 4 (codons 117 and 146). The weight of current evidence indicates that anti-EGFR monoclonal antibody therapy should only be considered for treatment of individuals whose tumor is determined to not have mutations detected after such extended RAS testing.

What's New and Different?

In addition to testing for mutations in KRAS exon 2 (codons 12 and 13) as recommended previously, before treatment with anti-EGFR antibody therapy, individuals with mCRC should have their tumor tested for mutations in:

- KRAS exons 3 (codons 59 and 61) and 4 (codons 117 and 146)
- NRAS exons 2 (codons 12 and 13), 3 (codons 59 and 61), and 4 (codons 117 and 146)

Targeted therapies such as anti-VEGF and anti-EGFR agents may be added to doublet chemotherapies in maximal settings. […] If molecular testing results for *RAS* (*KRAS/NRAS*) are available, this guideline provides recommendations according to the status of these markers. In maximal (-resource) settings, for individuals with left-sided colon cancer and known *KRAS/NRAS* wild type (WT) molecular status, anti-EGFR antibodies such as cetuximab or panitumumab may be added to chemotherapy doublet, with a moderate-strength recommendation. However, individuals with right-sided colon cancer and *RAS* WT status should not be offered treatment with anti-EGFR antibodies in the first-line setting. Anti-EGFR therapies have increased response rates and conversion from unresectable to resectable metastatic disease when added to chemotherapy with FOLFOC or FOLFIRI for individuals with *RAS* wildtype, but more recent data suggest that debenit with anti-EGFR therapies seems to be limited to individuals whose primary tumors are left-sided.

Please review the complete therapeutic recommendations that are located here: (2 , 23)

2020 Clinical Practice Guidelines in Oncology: Colon Cancer, from the National Comprehensive Cancer Network (NCCN)

Version 4.2020 – Discussion update in progress.

A sizable body of literature has shown that tumors with a mutation in codon 12 or 13 of exon 2 of the *KRAS* gene are essentially insensitive to cetuximab or panitumumab therapy. More recent evidence shows mutations in *KRAS* outside of exon 2 and mutations in *NRAS* are also predictive for a lack of benefit to cetuximab and panitumumab.

The panel therefore strongly recommends *RAS* (*KRAS/NRAS)* genotyping of tumor tissue (either primary tumor or metastasis) in all individuals with metastatic colorectal cancer. Individuals with known *KRAS* or *NRAS* mutations should not be treated with either cetuximab or panitumumab, either alone or in combination with other anticancer agents, because they have virtually no chance of benefit and the exposure to toxicity and expense cannot be justified. It is implied throughout the guidelines that NCCN recommendations involving cetuximab or panitumumab relate only to individuals with disease characterized by *KRAS/NRAS* wild-type genes. ASCO released a Provisional Clinical Opinion Update on extended *RAS* testing in individuals with metastatic colorectal cancer that is consistent with the NCCN panel's recommendations. A guideline on molecular biomarkers for colorectal cancer developed by the ASCP, CAP, AMP and ASCO also recommends *RAS* testing consistent with the NCCN recommendations.

The recommendation for KRAS/NRAS testing, at this point, is not meant to indicate a preference regarding regimen selection in the first-line setting. Rather, this early establishment of KRAS/NRAS status is appropriate to plan for the treatment continuum, so that the information may be obtained in a non- time–sensitive manner and the individual and provider can discuss the implications of a KRAS/NRAS mutation, if present, while other

treatment options still exist. Note that because anti-EGFR agents have no role in the management of stage I, II, or III disease, KRAS/NRAS genotyping of colorectal cancers at these earlier stages is not recommended. […] The NCCN Colon/Rectal Cancer Panel believes that *RAS* mutation status should be determined at diagnosis of stage IV disease. Individuals with any known *RAS* mutation should not be treated with either cetuximab or panitumumab.

KRAS mutations are early events in colorectal cancer formation, and therefore a very tight correlation exists between mutation status in the primary tumor and the metastases. For this reason, *KRAS/NRAS* genotyping can be performed on archived specimens of either the primary tumor or a metastasis. Fresh biopsies should not be obtained solely for the purpose of *KRAS/NRAS* genotyping unless an archived specimen from either the primary tumor or a metastasis is unavailable.

Approximately 5% to 9% of colorectal cancers are characterized by a specific mutation in the *BRAF* gene (V600E). *BRAF* mutations are, for all practical purposes, limited to tumors that do not have *KRAS* exon 2 mutations. Activation of the protein product of the non-mutated *BRAF* gene occurs downstream of the activated KRAS protein in the EGFR pathway. The mutated BRAF protein product is believed to be constitutively active, thereby putatively bypassing inhibition of EGFR by cetuximab or panitumumab.

The panel recommends that *KRAS*, *NRAS*, and *BRAF* gene testing be performed only in laboratories that are certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA-88) as qualified to perform highly complex molecular pathology testing. No specific testing methodology is recommended.

Please review the complete therapeutic recommendations that are located here:(3)

Allele Nomenclature

Selected *KRAS* Somatic Variants

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
G12D	p.Gly12Asp	NM_004985.5:c.35G>A	NP_004976.2:p.Gly12Asp	rs121913529
G12V	p.Gly12Val	NM_004985.5:c.35G>T	NP_004976.2:p.Gly12Val	rs121913529
G13D	p.Gly13Asp	NM_033360.4:c.38G>A	NP_004976.2:p.Gly13Asp	rs112445441

Selected *NRAS* Somatic Variants

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
NRAS G12V	p.Gly12Val	NM_002524.5:c.35G>T	NP_002515.1:p.Gly12Val	rs121913237
NRAS G13R	p.Gly13Arg	NM_002524.5:c.37G>C	NP_002515.1:p.Gly13Arg	rs121434595
NRAS Q61R	p.Gln61Arg	NM_002524.5:c.182A>G	NP_002515.1:p.Gln61Arg	rs11554290
NRAS Q61K	p.Gln61Lys	NM_002524.5:c.181C>A	NP_002515.1:p.Gln61Lys	rs121913254

Selected *BRAF* Somatic Variants

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
V600E	p.Val600Glu	NM_004333.6:c.1799T>C	NP_004324.2:p.Val600Glu	rs113488022

Guidelines for the description and nomenclature of gene variations are available from the Human Genome Variation Society (HGVS).

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