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# Cetuximab Therapy and *RAS* and *BRAF* Genotype

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## Introduction

Cetuximab (brand name Erbitux) is a monoclonal antibody used in the treatment of metastatic colorectal cancer (mCRC) and cancer of the head and neck. Cetuximab is an epidermal growth factor receptor (EGFR) antagonist, which works by blocking the growth of cancer cells. It is administered as a weekly intravenous (IV) infusion, but in practice, is often given every other week to coincide with chemotherapy (for example, FOLFIRI or FOLFOX). Cetuximab has several off-label uses as well, which include non-small cell lung cancer, squamous cell carcinoma of the skin, and Menetrier's disease.

Interestingly, for colorectal cancer, the location of the primary tumor influences whether an individual with mCRC will respond to anti-EGFR therapy, and influences prognosis. 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, currently only the mutation status of the tumor, and not the location of the tumor, is discussed in the drug label's dosing recommendations.

Resistance to cetuximab is associated with specific *RAS* mutations. The *RAS* family of oncogenes includes the *KRAS* and *NRAS* genes. When mutated, these genes have the ability to transform normal cells into cancerous 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 mitogen-activated protein kinase (MAPK) pathway and are associated with resistance to anti-EGFR drugs such as cetuximab. In addition, mutations in *NRAS* and another gene, *BRAF*, have been associated with poor response to anti-EGFR therapy; however, *BRAF* mutation does not explicitly preclude anti-EGFR therapy. Combination therapies targeting both BRAF and EGFR have shown to improve survival for individuals with wild-type *RAS* and mutant *BRAF*.

The 2018 FDA-approved drug label for cetuximab states that for mCRC, cetuximab is indicated for *K*- and *N*-*RAS* wild-type (no mutation), *EGFR*-expressing tumors. The label states that an FDA-approved test must be used to confirm the absence of a *RAS* mutation (in either *KRAS* or *NRAS*) prior to starting cetuximab (Table 1) (1). While the FDA label also states that EGFR expression should also be confirmed by an approved test prior to starting therapy for mCRC, this is largely not implemented in practice, nor is it recommended by professional oncology society guidelines.

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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 mutations detected after extended *RAS* testing (Table 2) (2).

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 cetuximab (and panitumumab) highly unlikely unless given a BRAF inhibitor (Table 3) (3).

**Table 1.** The FDA-Approved Cetuximab Label: Dosage and Administration (2020)

| Genes to be tested | Recommendations for metastatic colorectal cancer |
|---|---|
| *NRAS*<br>*KRAS* | Cetuximab 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 *K-Ras* or *N- Ras* and hereafter is referred to as "*RAS*" or when the Ras status is unknown.<br>Confirm the absence of a *RAS* mutation prior to initiation of treatment with cetuximab.<br>Information on FDA-approved tests for the detection of *K-Ras* mutations in individual with metastatic colorectal cancer is available here. |
| *EGFR* | Determine EGFR expression status using FDA-approved tests prior to initiating treatment with cetuximab. |

This table is created from (1). *EGFR,* epidermal growth factor receptor

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

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

This 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 in Metastatic Colorectal Cancer (2020)

| Genes to be tested | Recommendations for colorectal cancer |
|---|---|
| *KRAS*<br>*NRAS* | All individuals with metastatic colorectal cancer should have tumor tissue genotyped for *RAS* (*KRAS and NRAS*) and *BRAF* mutations.<br>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 table is created from (3). NCCN, National Comprehensive Cancer Network

# Drug: Cetuximab

Cetuximab is an EGFR antagonist. It is used for the treatment of mCRC, and squamous cell carcinoma of the head and neck. Cetuximab, and the related drug panitumumab (brand name Vectibix, approved only for

mCRC), 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). Cetuximab has also been used for off-label indications that include non-small cell lung cancer (5), squamous cell carcinoma of the skin (6), and Menetrier's disease (7).

Cetuximab is a chimeric monoclonal antibody composed of regions of both mouse and human antibody, whereas panitumumab is a fully human monoclonal antibody. Both biological agents have been shown to provide a clear clinical benefit in the treatment of *RAS* wild-type mCRC (8, 9).

Colorectal cancer is the third leading cause of cancer death for men and women in the US, and the second in Europe (10). Radiation therapy is generally used for early stage rectal cancer. Surgery is the most common treatment for localized colorectal cancer that has not spread, and chemotherapy 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 (11).

Treatment regimens for advanced or metastatic colorectal carcinoma include chemotherapy such as folinic acid, fluorouracil, irinotecan, capecitabine, and oxaliplatin. Targeted biological agents may be added to such regimens, such as cetuximab, panitumumab, and bevacizumab. Bevacizumab (brand name Avastin) is a monoclonal antibody that targets vascular endothelial growth factor, VEGF. Similar FDA-approved biologics include aflibercept (a VEGF inhibitor monoclonal antibody) and regorafenib (a receptor tyrosine kinase inhibitor with activity against VEGF receptors).

Cetuximab is used in combination with FOLFIRI (FOLinic acid, Fluorouracil, IRInotecan) or FOLFOX (FOLinic acid, Fluorouracil, Oxaliplatin) for first-line treatment; or in combination with irinotecan in individuals who are refractory to irinotecan-based chemotherapy (1, 12). Cetuximab may also be used as a single agent (monotherapy) in individuals who either did not respond to oxaliplatin- and irinotecan-based chemotherapy or are intolerant to irinotecan (3).

Interestingly, 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) (13). These differences in embryologic origin correlate with common genetic alterations. Right-sided tumors are more likely to have mutated *RAS* and *BRAF*, while left-sided tumors may have upregulated *EGFR* and/or *ERBB2* (*HER2*) (14). Thus, individuals with left-sided tumors benefit more from EGFR therapy than individuals with right-sided tumors (15, 16, 17).

Multiple professional guidelines suggest that cetuximab has limited benefit in first-line therapy for right-sided tumors (18, 19). Right-sided tumors may respond to bevacizumab in combination with chemotherapy, with potentially longer overall survival compared with cetuximab combination treatment (15, 16, 20). A recent review highlighted multiple retrospective studies regarding the prognostic and predictive power of right- versus left-sidedness of the primary tumor. The authors concluded that in first-line treatment, left-sided tumors were distinctly more likely to respond to anti-EGFR treatment. However, there was no clear consensus for the implications of tumor sidedness with respect to second-line (and beyond) treatment. (21)

Administration of IV anti-EGFR therapy may be associated with severe infusion reactions, including anaphylaxis (3% for cetuximab and 1% for panitumumab) and these reactions are more common in cetuximab treatment versus panitumumab (22). Other toxicities include cardiopulmonary arrest, severe skin rashes (the severity of which may predict an increased response and survival, regardless of *RAS* mutational status (23, 24)), and an increased risk of venous thrombosis and embolism (2, 11, 25). Additionally, a higher rate of cetuximab-induced infusion reactions has been reported in head and neck cancer treatment (5.4%) as compared with mCRC treatment (26). Within the United States, there appears to be a higher risk of anaphylaxis reaction in

areas of the Southeast including North Carolina, Virginia, Tennessee, Florida as well as Missouri and Kansas (reviewed by (27)). Evidence suggests these infusion reactions are due to the presence of immunoglobulin E antibodies targeting a specific glycosylation moiety found on cetuximab (28). The presence of these antibodies in cetuximab-naïve individuals may be due to prior bites from the lone star tick (Amblyomma americanum) *(29)*.

Cetuximab can cause fetal harm when administered during pregnancy. There are no studies in pregnant women, but in animal studies (cynomolgus monkeys) the administration of IV cetuximab in pregnancy resulted in an increased risk of fetal death. Women should be advised to use effective contraception during cetuximab therapy and for 2 months after the last dose. Women should also be advised of the potential risks to the fetus, and to inform their healthcare provider if they know or suspect they are pregnant.

An important role in the progression of mCRC is thought to involve the impaired regulation of EGFR function, resulting in activation of the associated MAPK pathway. Cetuximab and panitumumab are important agents in metastatic disease because they can block the activation of the MAPK pathway. However, resistance to these agents can arise through constitutive activation of the MAPK pathway, which is caused by mutations in downstream signaling proteins, such as KRAS, NRAS and BRAF. Approximately 40% of cases of mCRC are found to have activating mutations in *KRAS*.

The efficacy of cetuximab 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 mutations in the same exons—2, 3, and 4—are also associated with a lack of response to cetuximab (17, 30, 31, 32, 33).

Therefore, expanded *RAS* testing (of *KRAS* and *NRAS*) is the standard of care to determine which individuals with mCRC may benefit from anti-EGFR therapy (34, 35).

Epidermal growth factor receptor overexpression is seen commonly in squamous cell head and neck cancers and is associated with poor survival outcomes (36, 37). Trials with cetuximab in local or metastatic head and neck cancers have shown this anti-EGFR therapy to have most benefit as part of combination therapies in metastatic cancer, with limited application in local, unresectable disease (reviewed by (18)). Neither the FDA-approved drug label nor NCCN guidelines recommend RAS genetic testing before initiating therapy with cetuximab in head and neck cancers (1, 18).

## 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 (11). Members of the *RAS* family and the *EGFR* gene are all proto-oncogenes.

The *RAS* family contains three 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.

Mutations 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.

The *EGFR* gene is a member of the human epidermal growth factor receptors family (HER) along with *ERBB2* (*HER2)*, *ERBB3* (*HER3)* and *ERBB4* (*HER4)*. Overexpression of these genes has been associated with multiple cancer types. These receptors dimerize upon binding of an extracellular ligand and activate the downstream signaling pathways, including Ras/Raf/Mek/Erk proteins. In some contexts, overexpression of HER2 can promote dimerization without an extracellular signal, leading to constitutive activation. (38, 39)

## Gene: *KRAS*

*KRAS* 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 40% of all *RAS* mutations in mCRC (40). Individuals with mCRC that harbor *KRAS* mutations do not benefit from anti–EGFR therapy (either cetuximab or panitumumab therapy) (3, 41, 42, 43, 44, 45, 46).

## Gene: *NRAS*

*NRAS* 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, 47, 48, 49).

Individuals with *NRAS*-mutated tumors are less likely to respond to cetuximab or panitumumab (19, 25). Panitumumab may even have a detrimental effect in individuals with *NRAS* or *KRAS* mutations (2).

## Gene: *BRAF*

RAF is 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 (50).

*BRAF* mutations are detectable in approximately 5–15% of mCRCs. They tend to only occur in tumors that do not have *KRAS* exon 2 mutations (51). 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) (52).

By far the most common *BRAF* mutation is known as V600E, which accounts for approximately 90% of *BRAF* mutations. The mutated BRAF protein is constitutively active and is a highly potent oncogene, acting downstream in the EGFR pathway, thus bypassing inhibition of EGFR by cetuximab or panitumumab (10). 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 (53).

The *BRAF* V600E mutation is associated with a poorer prognosis 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 (54). Alternative treatments may include the use of drug combinations, such as the addition of a BRAF inhibitor to anti-EGFR, to overcome resistance (35, 55). Indeed, utilization of BRAF inhibitor therapy in combination 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 (54, 56). 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 colorectal cancer 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 in combination 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 HER family consists of 4 members: *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 protein is expressed in many different tissues, and is activated by the binding of a ligand, such as epithelial growth factor (EGF) or transforming growth factor α (TGFα). 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 EGFR a target for anticancer drugs (57, 58, 59).

Currently, 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 protein is overexpressed in several cancers, including squamous cell carcinoma of the head and neck, squamous cell lung cancer, and colorectal cancer. The EGFR protein is overexpressed in approximately 50–80% of colorectal tumors (2, 60). The FDA-approved drug label for cetuximab states that the drug is licensed for EGFR-expressing mCRC, and mentions in animal studies using human tumor xenografts that lacked EGFR expression, that no anti-tumor effects of cetuximab were observed (1). However, for colorectal cancer, EGFR expression has not been associated with efficacy of anti-EGFR therapy (61).

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 cetuximab or panitumumab. 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: *ERBB2 / HER2*

HER2 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 cetuximab response (35, 62) A recent review of HER2 retrospective studies found a consistent correlation between *ERBB2* amplification and resistance to anti-EGFR treatment (38).

The NCCN Colon/Rectal Cancer Panel recommends *ERBB2* amplification/overexpression testing for individuals with mCRC. However, if the tumor is known to have a *RAS* or *BRAF* mutation, *ERBB2* testing is not required.

Based on the outcome of HER2 expression 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 cetuximab (3). A dual tyrosine kinase inhibitor targeting HER2 and EGFR called lapatinib is also available and can be used in combination with anti-HER2 monoclonal antibodies for *ERBB2*-amplified mCRC (3).

## Linking gene variation with treatment response

It has been established that specific variants in the genes *KRAS* and *NRAS* result in resistance to cetuximab therapy. In addition, the presence of the *BRAF* V600E mutation makes a beneficial response to treatment unlikely, unless given with a BRAF inhibitor (3, 56, 63). Specific point-mutation variants in *ERBB2* and *EGFR* do not appear to be associated with cetuximab resistance. However, *ERBB2* overexpression has been associated with decreased success of anti-EGFR therapies (38, 39).

## Genetic Testing

The NIH Genetic Testing Registry, GTR, displays genetic tests that are available for the cetuximab drug response, and the genes *KRAS*, *NRAS*, *EGFR*, *BRAF* and *ERBB2*.

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

**KRAS, NRAS, and BRAF Mutation and HER2 Testing**

- 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 V600E mutation makes response to panitumumab or cetuximab highly unlikely unless given with a BRAF inhibitor. WT RAS/BRAF tumors should also be screened for HER2 overexpression/amplification.
- 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 with 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.**

## 2020 Statement from the US Food and Drug Administration (FDA)

**2.2 Recommended Dosage for Colorectal Cancer (CRC)**

Determine EGFR-expression status using FDA-approved tests prior to initiating treatment. Also confirm the absence of a Ras mutation prior to initiation of treatment with cetuximab. Information on FDA-approved tests for the detection of K-Ras mutations in patients with metastatic CRC is available at: http://www.fda.gov/medicaldevices/productsandmedicalprocedures/invitrodiagnostics/ucm301431.htm.

[...]

**5.7 Increased Tumor Progression, Increased Mortality, or Lack of Benefit in Patients with Ras- Mutant mCRC**

Cetuximab is not indicated for the treatment of patients with CRC 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 K-Ras or N- Ras and hereafter is referred to as "Ras" or when the Ras status is unknown.

Retrospective subset analyses of Ras-mutant and wild-type populations across several randomized clinical trials, including CRYSTAL, were conducted to investigate the role of Ras mutations on the clinical effects of anti-EGFR-directed monoclonal antibodies. Use of cetuximab in patients with Ras mutations resulted in no clinical benefit with treatment related toxicity. Confirm Ras mutation status in tumor specimens prior to initiating cetuximab.

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

## 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 patients with metastatic colorectal cancer. Patients 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

[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. Certain terms, genes and genetic variants may be corrected in accordance with nomenclature standards, where necessary. We have given the full name of abbreviations, shown in square brackets, where necessary.

cannot be justified. ASCO released a Provisional Clinical Opinion Update on extended *RAS* testing in patients with metastatic colorectal cancer (mCRC) that is consistent with the NCCN panel's recommendations. A guideline on molecular biomarkers for CRC developed by the ASCP, CAP, AMP and ASCO also recommends resting consistent with the NCCN recommendations.

The recommendation for *RAS* testing, at this point, is not meant to indicate a preference regarding regimen selection in the first-line setting. Rather, this early establishment of *RAS* status is appropriate to plan for the treatment continuum, so that the information may be obtained in a non- time–sensitive manner and the patient and provider can discuss the implications of a *RAS* 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, *RAS* genotyping of colorectal cancers at these earlier stages is not recommended.

*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, *RAS* 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 *RAS* genotyping unless an archived specimen from either the primary tumor or a metastasis is unavailable.

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. The three genes can be tested individually or as part of an NGS panel.

[…]

HER2 is a member of the same family of signalling kinase receptors as EGFR and has been successfully targeted in breast cancer in both the advanced and adjuvant settings. HER2 is rarely amplified/overexpressed in CRC (approximately 3% overall), but the prevalence is higher in *RAF/BRAF*-wild type tumors (reported at %5-14%). Specific molecular diagnostic methods have been proposed for HER2 testing in CRC and HER2-targeted therapies are now recommended as subsequent therapy options in patients with tumors that have HER2 overexpression. Based on this, the NCCN Guidelines recommend testing for HER2 amplifications for patients with mCRC. If the tumor is already known to have a *KRAS/NRAS* or *BRAF* mutations, HER2 testing is not required. As HER2-targeted therapies are still under investigation, enrollment in a clinical trial is encouraged.

Evidence does not support a prognostic role of HER2 overexpression. In addition to its role as a predictive marker for HER2-targeted therapy, initial results indicated HER2 amplification/overexpression may be predictive of resistance to EGFR-targeting monoclonal antibodies.

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

2015 Provisional Clinical Opinion from the American Society of Clinical Oncology (ASCO)
All patients with metastatic colorectal cancer who are candidates for anti-EGFR antibody therapy should 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 MoAb therapy should only be considered for treatment of patients 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, patients 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)

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

# 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_004985.4:c.35G>A | NP_004976.2:p.Gly12Asp | rs121913529 |
| *G12V* | p.Gly12Val | NM_004985.4:c.35G>T | NP_004976.2:p.Gly12Val | rs121913529 |
| *G13D* | p.Gly13Asp | NM_033360.3: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_002524.4:c.35G>T | NP_002515.1:p.Gly12Val | rs121913237 |
| *NRAS G13R* | p.Gly13Arg | NM_002524.4:c.37G>C | NP_002515.1:p.Gly13Arg | rs121434595 |
| *NRAS Q61R* | p.Gln61Arg | NM_002524.4:c.182A>G | NP_002515.1:p.Gln61Arg | rs11554290 |
| *NRAS Q61K* | p.Gln61Lys | NM_002524.4: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_004333.4: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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