OptimaHealth
A Service of Sentara

KRAS, *NRAS* and *BRAF* Mutation Analysis in Colorectal Cancer

Policy Number: AHS – M2026 – *KRAS, NRAS* and *BRAF* Mutation Analysis in Colorectal Cancer	• Prior Policy Name and Number, as applicable: AHS-M2026-*KRAS* and *BRAF* Mutation Analysis in Colorectal Cancer
Initial Presentation Date: 06/16/2015 Revision Date: 06/02/2021	

I. Policy Description

Monoclonal antibodies that bind the epidermal growth factor receptor (*EGFR*), such as cetuximab, and block its activation have led to significant clinical benefits for metastatic colorectal cancer (mCRC) patients (De Roock et al., 2010). Mutations in downstream effectors of the *EGFR* pathway have been associated with resistance to *EGFR* antibody chemotherapies (Allegra et al., 2009; Compton, 2020; Sepulveda et al., 2017).

II. Related Policies

Policy Number	Policy Title
AHS-M2004	Lynch Syndrome
AHS-M2024	Genetic Testing for Polyposis Syndromes
AHS-M2066	Genetic Cancer Susceptibility Using Next Generation Sequencing
AHS-M2109	Molecular Panel Testing Of Cancers To Identify Targeted Therapy

III. Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. If there is a conflict between this Policy and any relevant, applicable government policy [e.g. Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare] for a particular member, then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit their search website http://www.cms.gov/medicare-coverage-database/overview-and-quick-search.aspx or the manual website

1. Tumor tissue genotyping for Kirsten rat sarcoma virus (*KRAS),* Neuroblastoma RAS viral oncogene homolog *(NRAS)* and B-Raf proto-oncogene (*BRAF)* mutations **MEETS COVERAGE CRITERIA** for all patients with metastatic colorectal cancer.

2. Testing for *KRAS* mutation (exon 2, 3, 4), *NRAS* (exon 2, 3, 4) and *BRAF* V600 mutations **MEETS COVERAGE CRITERIA** prior to deciding treatment with cetuximab or panitumumab.

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of a patient's illness.

3. Testing for *KRAS, NRAS* and *BRAF* V600 mutations in all other situations not described above **DOES NOT MEET COVERAGE CRITERIA**

NOTE: For 5 or more gene tests being run on a tumor specimen (i.e. non-liquid biopsy) on the same platform, such as multi-gene panel next generation sequencing, please refer to AHS-R2162 Reimbursement Policy.

IV. Scientific Background

Colorectal cancer (CRC) is the second leading cause of cancer-related deaths in the United States following lung cancer. 20% of patients with colorectal cancer will present with metastatic colorectal cancer (mCRC) at diagnosis and a significantly poorer prognosis. The 5-year survival is 13.1% in patients with distant metastases from CRC as compared to 64.9% for all CRC patients (El-Deiry et al., 2015).

Certain mutations may affect treatment of CRC. For example, the activation of the epidermal growth factor receptor (*EGFR*) signaling cascade is associated with colon tumorigenesis (Therkildsen, Bergmann, Henrichsen-Schnack, Ladelund, & Nilbert, 2014); therefore, medications such as cetuximab or panitumumab that target the *EGFR* pathway may be used in treatment of CRC. However, activating mutations in the *KRAS* oncogene will cause anti-*EGFR* resistance since these mutations can result in a constitutively active pathway, even with anti-*EGFR* treatment (Clark & Grothey, 2021). Consequently, tumors with mutated *KRAS* are unresponsive to anti-*EGFR* therapy. As a result, testing for mutational status as a negative predictive factor for anti-*EGFR* therapy has become part of routine pathological evaluation for CRC. Other mutations in the RAS oncogene (primarily *NRAS*) may also lead to the same phenotype (Frucht & Lucas, 2021). Another gene that may be overexpressed within the *EGFR* pathway is *HER2* (human epidermal growth factor receptor 2). This gene plays a role in activating signal transduction pathways controlling epithelial cell growth. Although *HER2* is more traditionally known as a breast cancer-associated gene, up to 5% of colorectal cancer cases are found to overexpress *HER2* (Clark & Grothey, 2021).

Another component of the RAS signaling pathway, *BRAF*, has also been found to affect anti-*EGFR* treatment. *BRAF* V600E mutations may also confer a lack of response to anti-*EGFR* treatment even when paired with a wild type RAS oncogene. Mutations in this region occur in less than 10% of sporadic CRCs, and the mutation at position 600 is the primary polymorphism found in CRC. Non-V600 *BRAF* mutations are rarer (composing about 2.2% of patients with metastatic CRC) and confer a generally better prognosis than their V600 mutated counterparts; a study found non-V600 genotypes to lead to better median overall survival and fewer high-grade tumors (Jones et al., 2017).

Clinical Validity and Utility

In a meta-analysis by Xu et al. (2013), a total of 2875 patients were evaluated, with 246 patients having *BRAF* mutations. The objective response rate (ORR) to *EGFR* therapy was 18.4% (40/217) in mutant *BRAF* group and 41.7% (831/1993) in the wild-type *BRAF* group. The overall risk ratio for the ORR of *BRAF* mutations compared to wild-type *BRAF* patients was 0.58. The median progression free survival (hazard ratio 2.98) and overall survival (hazard ratio: 2.85) were significantly shorter of patients with *BRAF* mutations compared to patients with wild-type *BRAF* mutations (Xu et al., 2013).

Douillard et al. (2013) evaluated the effect of panitumumab plus oxaliplatin, fluorouracil, and leucovorin (FOLFOX4) compared to just FOLFOX4 on patients with varying RAS and *BRAF* mutations. 639 patients with metastatic CRC without mutations in *KRAS* exon 2 had at least one of the following: *KRAS* exon 3 or 4; *NRAS* exon 2, 3, or 4; or *BRAF* exon 15. 228 patients had neither RAS nor *BRAF* mutations, and this group was evaluated to have better survival metrics with panitumumab plus FOLFOX4 than the group with just FOLFOX4 (median of 10.8 months progression-free survival and 28.3 months overall survival for panitumumab group vs 9.2 and 20.9 respectively for the group without). However, 296 patients with either a *RAS* or *BRAF* mutation were treated with panitumumab plus FOLFOX4, and this group's survival metrics were lower than the group only treated with FOLFOX4. The *RAS*/*BRAF* group treated with panitumumab plus FOLFOX4 had a median of only 7.3 months progression-free survival and 15.3 months overall survival vs 8.0 and 18.0 for the 305 patients treated with only FOLFOX4). The authors concluded that additional RAS mutations predicted a lack of response to panitumumab plus FOLFOX4 (Douillard et al., 2013).

Therkildsen et al. (2014) performed a meta-analysis of the clinical impact of anti-*EGFR* treatment on patients with *KRAS*, *NRAS*, and *BRAF* mutations (as well as PIK3CA and PTEN). 22 studies including 2395 patients were evaluated. Odds ratios for objective response rate (ORR) and hazard ratios (HR) for progression-free survival rate (PFS) and overall survival (OS) were calculated. Mutations in *KRAS* exons 3 and 4 and *BRAF* predicted poor ORR (0.26 and 0.29 respectively), *KRAS*, *NRAS*, and *BRAF* mutations all led to significantly lower progression-free survival (HR = 2.19, 2.30, and 2.95 respectively) and significantly lower overall survival (HR = 1.78, 1.85, and 2.52 respectively) (Therkildsen et al., 2014).

Rebersek et al. (2019) investigated the impact of molecular biomarkers on survival and response to first line therapy in metastatic colorectal cancer patients. 154 patients were included, with 42% harboring *KRAS* mutations and 3% harboring *BRAF* mutations. Median overall survival (OS) was found to be 56.5 months for wild-type *KRAS* patients and 58 months for mutated *KRAS* patients. Median OS for mutated exon 12 patients was 57 months compared to 44 months for mutated exon 13 patients. Wild-type *KRAS* was found to affect the response to first-line systemic therapy, whereas no other parameters were found to affect response (Rebersek, Mesti, Boc, & Ocvirk, 2019).

Sartore-Bianchi et al. (2019) investigated the effect of *HER2* positivity on anti-*EGFR* treatment. 100 patients HER2-positive (of 1485 wild-type *KRAS* exon 2 patients) with metastatic colorectal cancer were included. The authors found that HER2-positive patients had more frequent lung metastases (odds ratio [OR] = 2.04) and higher tumor burden (OR = 1.48). The 79 *HER2*-positive patients given anti-*EGFR* treatment were also found to have poorer clinical outcomes, with lower objective response rate (31.2% compared to 46.9% for all others) and lower progression-free survival (5.7 months vs 7 months). The authors concluded that HER2 testing should be offered because "occurrence of this biomarker is unlikely to be predicted based on main clinicopathological features" (Sartore-Bianchi et al., 2019).

Cenaj et al. (2019) evaluated the correlation between "*ERBB2* amplification by next-generation sequencing (NGS) with HER2 overexpression by immunohistochemistry". NGS was performed on specimens with 20% or more tumor, and 1300 cases of colorectal cancer were included. *ERBB2* amplification was detected in 2% of cases. HER2 amplification was examined in "15 cases with *ERBB2* amplification (six or more copies), 10 with low gain (three to five copies), and 77 copy neutral". *ERBB2* amplification was found to have perfect concordance with HER2 immunochemistry at H-scores of 105 or more. Further, *ERBB2* amplification was found to inversely correlate with RAS/RAF mutations. The authors concluded that "NGS-detected *ERBB2* amplification highly correlates with HER2 overexpression in CRC", which may support authors' original hypothesis that ERBB2 amplification/overexpression may predict response to HER2 inhibitors (Cenaj, Ligon, Hornick, & Sholl, 2019).

Fan et al. (2021) analyzed the relationship between mismatch repair (MMR) protein, *RAS*, *BRAF,* and *PIK3CA* expression and clinicopathological characteristics in elderly patients with CRC. From 327 patients, the researchers found that "the mutation rates of the *KRAS, NRAS, BRAF* and *PIK3CA* genes in elderly CRC patients were 44.95% (147/327), 2.45% (8/327), 3.36% (11/327) and 2.75% (9/327), respectively." They also identified that "*KRAS* was closely related to tumor morphology ($P = 0.002$) but not to other clinicopathological features ($P > 0.05$), and there were no significant differences between *NRAS* gene mutation and clinicopathological features ($P > 0.05$). The *BRAF* gene mutation showed a significant difference in pathological type, tumor location, differentiation degree and lymph node metastasis ($P < 0.05$), but was not correlated with sex, tumor size and tumor morphology ($P > 0.05$)" (Fan et al., 2021). This demonstrates the critical nature of mutation analysis for these specific genes to aid in identifying potential therapies that would better patient prognoses especially in such a vulnerable population like the elderly.

The prognostic benefit was corroborated by Chang et al. (2021), who found that the *BRAF* gene mutation was "associated with cancer thrombosis in blood vessels" and was "negatively correlated with the OS [overall survival] rate of CRC patients" in their patient population (n=410) from Central China. Like Fan et al. (2021), *KRAS* also had the greatest mutation rate at 47.56% in this study, showing more awareness needed for tissue genotyping for mCRC (Chang et al., 2021).

Formica et al. (2020) examined tumor tissue (T) mutational analysis in terms of discordance with circulating tumor DNA (ctDNA) obtained by liquid biopsy from plasma (PL) and assessed through real time polymerase chain reaction (PCR). Despite finding concordance for patients with *BRAF* mutations between the tissue and plasma samples, 20% of patients were *RAS* discordant. Mutations identified from ctDNA were able to refine the prognosis determined by tissue samples – "*RAS* wild type in T and mutated in PL had significantly shorter PFS than concordant *RAS* wild type in T and PL: mPFS [median progression free survival] 9.6 vs. 23.3 months, respectively, $p = 0.02$. Patients RAS mutated in T and wild type in PL had longer PFS than concordant RAS mutated in T and PL: 24.4 vs. 7.8 months, respectively, $p = 0.008$." This raises a limitation to using tumor tissue as the mainstay for mutational analysis and considering combining with or replacing tumor tissue genotyping with plasma ctDNA as a measure of prognosis going forward (Formica et al., 2020)

V. Guidelines and Recommendations

Food and Drug Administration (FDA, 2009, 2012)

As per FDA requirements, the Erbitux (cetuximab) package insert (FDA, 2012) indicates that the drug is to be used for "K-Ras mutation-negative (wild-type), *EGFR*-expressing, metastatic colorectal cancer as determined by FDA-approved tests." Similarly, the Vectibix (panitumumab) package insert (FDA, 2009) states that "Use of Vectibix is not recommended for the treatment of colorectal cancer with these [*KRAS*] mutations ."

American Society of Clinical Oncology (ASCO) (Chiorean et al., 2020; Sepulveda et al., 2017)

ASCO published a Provisional Clinical Opinion (PCO) that states "*RAS* mutational testing of colorectal carcinoma tissue should be performed in a Clinical Laboratory Improvement Amendments–certified laboratory for all patients who are being considered for anti-*EGFR* MoAb therapy". ASCO recommends that "mutational analysis should include *KRAS* and *NRAS* codons 12 and 13 of exon 2; 59 and 61 of exon 3; and 117 and 146 of exon 4. The weight of current evidence indicates that anti-*EGFR* MoAb therapy (currently cetuximab and panitumumab) should only be considered for treatment of patients with mCRC who are identified as having tumors with no mutations detected after such extended RAS mutation analysis" (Allegra et al., 2016).

This guideline was archived and replaced by Sepulveda et al. (2017) (ASCO).

In 2020, ASCO published a guideline titled "Treatment of Patients With Late-Stage Colorectal Cancer". ASCO recommends that all patients with mCRC should be tested for key molecular markers (when possible) if targeted treatments are available. *RAS* and *BRAF* are mentioned as examples of molecular markers (Chiorean et al., 2020).

National Comprehensive Cancer Network (NCCN, 2021)

The guidelines v.2.2021 recommend that "all patients with metastatic colorectal cancer should have tumor tissue genotyped for *RAS* (*KRAS* and *NRAS*) and *BRAF* mutations individually or as part of an NGS panel. Patients 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."

The NCCN guidelines state that testing for *KRAS*, *NRAS* and *BRAF* mutations should be performed only in laboratories that are CLIA-1988 certified as qualified to perform high complexity clinical laboratory (molecular pathology) testing. No specific methodology is recommended (e.g. sequencing, hybridization).

The NCCN further states that "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."

BRAF genotyping of tumor tissue is recommended at stage IV disease. Allele-specific polymerase-chain reaction (PCR), NGS, or immunohistochemistry (IHC) may be used to determine *BRAF* status.

The NCCN notes that *HER2* may be overexpressed in *RAS*/*BRAF* wild-type tumors. *HER2*-targeted therapies are now recommended in patients with HER2 overexpression. Therefore, the NCCN now recommends testing for *HER2* amplifications in patients with metastatic CRC. However, *HER2* testing is not required in patients with known *KRAS*/*NRAS* or *BRAF* mutations, and the NCCN states that anti

HER2 therapy is only indicated in HER2-positive tumors that are also *RAS* and *BRAF* wild type (NCCN, 2021).

Routine *EGFR* testing is not recommended (NCCN, 2021).

Overall, the NCCN states that "determination of tumor gene status for *KRAS*/*RAS* and *BRAF* mutations, as well as *HER2* amplifications and MSI/MMR [microsatellite instability/mismatch repair] status (if not previously done), are recommended for patients with mCRC" (NCCN, 2021).

Evaluation of Genomic Applications in Practice and Prevention (EGAPP) Working Group (EWG)

The EWG determined that, "for patients with metastatic colorectal cancer (mCRC) who are being considered for treatment with cetuximab or panitumumab, there is convincing evidence to recommend clinical use of *KRAS* mutation analysis to determine which patients are *KRAS* mutation positive and therefore unlikely to benefit from these agents before initiation of therapy (EGAPP, 2013)." However, the EWG "found insufficient evidence to recommend for or against *BRAF* V600E testing for the same clinical scenario," and "the level of certainty for *BRAF* V600E testing to guide antiepidermal growth factor receptor (*EGFR*) therapy was deemed low" (EGAPP, 2013).

European Society for Medical Oncology (Argilés et al., 2020; Van Cutsem et al., 2016)

ESMO states that *RAS* mutational testing should be done at the time of diagnosing metastatic CRC and that RAS testing is mandatory before treatment with cetuximab and panitumumab. ESMO notes that *RAS* analysis should include "at least *KRAS* exons 2, 3 and 4 (codons 12, 13, 59, 61, 117 and 146) and *NRAS* exons 2, 3 and 4 (codons 12, 13, 59, 61 and 117)". ESMO also recommends that *BRAF* mutational status be assessed alongside *RAS* (Van Cutsem et al., 2016).

With regards to localized colon cancer, ESMO states that "besides MSI status, other genetic markers, e.g. *RAS* and *BRAF* mutations are not recommended for the routine assessment of risk of recurrence in non-metastatic patients, based on their lack of utility in the adjuvant decision-making process" (Argilés et al., 2020).

National Institute for Health and Care Excellence (NICE, 2020)

NICE recommends testing for *RAS* and *BRAF* V600E mutations in all people with metastatic colorectal cancer suitable for systemic anti-cancer treatment (NICE, 2020).

American Society for Clinical Pathology, College of American Pathologists, Association for Molecular Pathology, and the American Society of Clinical Oncology (Sepulveda et al., 2017)

These joint guidelines focus on "Molecular Biomarkers for the Evaluation of Colorectal Cancer". They list the following recommendations for *KRAS*, *NRAS*, and *BRAF* for CRC:

- "Colorectal carcinoma patients being considered for anti-*EGFR* therapy must receive RAS mutational testing. Mutational analysis should include *KRAS* and *NRAS* codons 12, 13 of exon 2; 59, 61 of exon 3; and 117 and 146 of exon 4 ("expanded" or "extended" RAS)".

- "*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".

- "There is insufficient evidence to recommend *BRAF* c.1799 p.V600 mutational status as a predictive molecular biomarker for response to anti-*EGFR* inhibitors" (Sepulveda et al., 2017).

VI. State and Federal Regulations, as applicable

A. Food and Drug Administration (FDA)

Cetuximab and panitumumab have FDA marketing approval for treatment of metastatic colorectal cancer in the refractory disease setting, and ongoing studies are investigating the use of these *EGFR* inhibitors as monotherapy and as part of combination therapy in first, second, and subsequent lines of therapy.

On May 23, 2014 the FDA approved therascreen *KRAS* RGQ PCR Kit is a real-time qualitative PCR assay used on the Rotor-Gene Q MDx instrument for the detection of seven somatic mutations in the human *KRAS* oncogene, using DNA extracted from formalin-fixed paraffin-embedded (FFPE), colorectal cancer (CRC) tissue. The therascreen *KRAS* RGQ PCR Kit is intended to aid in the identification of CRC patients for treatment with Erbitux (cetuximab) and Vectibix (panitumumab) based on a *KRAS* no mutation detected test result (FDA, 2014).

On May 7, 2015 the FDA approved cobas *KRAS* Mutation Test, for use with the cobas® 4800 System. Cobas is a real-time PCR test for the detection of seven somatic mutations in codons 12 and 13 of the *KRAS* gene in DNA derived from formalin-fixed paraffin-embedded human colorectal cancer (CRC) tumor tissue. The test is intended to be used as an aid in the identification of CRC patients for whom treatment with Erbitux (cetuximab) or with Vectibix (panitumumab) may be indicated based on a no mutation detected result (FDA, 2015).

On June 29, 2017 the FDA approved PraxisTM Extended RAS Panel as a qualitative in vitro diagnostic test using targeted high throughput parallel sequencing for the detection of 56 specific mutations in RAS genes [*KRAS* (exons 2, 3, and 4) and *NRAS* (exons 2, 3, and 4)] in DNA extracted from formalin-fixed, paraffin-embedded (FFPE) colorectal cancer (CRC) tissue samples. The Praxis™ Extended RAS Panel is indicated to aid in the identification of patients with colorectal cancer for treatment with Vectibix (panitumumab) based on a no mutation detected test result. The test is intended to be used on the Illumina MiSeqDx instrument (FDA, 2017).

On November 30, 2017, the FDA approved FoundationOne CDx, which is a next generation sequencing oncology panel. From the FDA website: "FoundationOne CDx™ (F1CDx) is a next generation sequencing based in vitro diagnostic device for detection of substitutions, insertion and deletion alterations (indels) and copy number alterations (CNAs) in 324 genes and select gene rearrangements, as well as genomic signatures including microsatellite instability (MSI) and tumor mutational burden (TMB) using DNA isolated from formalin-fixed paraffin embedded (FFPE) tumor tissue specimens. The test is intended as a companion diagnostic to identify patients who may benefit from treatment with the targeted therapies listed Table 1 in accordance with the approved therapeutic product labeling. Additionally, F1CDx is intended to provide tumor mutation profiling to be used by qualified health care professionals in accordance with professional guidelines in oncology for cancer patients with

solid malignant neoplasms. The F1CDx test is a single-site assay performed at Foundation Medicine, Inc." (FDA, 2017).

Additionally, many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). As an LDT, the U. S. Food and Drug Administration has not approved or cleared this test; however, FDA clearance or approval is not currently required for clinical use.

B. Centers for Medicare & Medicaid Services (CMS)

N/A

VII. Applicable CPT/HCPCS Procedure Codes

Code Number	Code Description
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)
81403	Molecular pathology procedure, Level 4 Gene: ***KRAS*** (v-Ki-ras2 Kirsten rat sarcoma viral oncogene) (eg, carcinoma), gene analysis , variant(s) in exon 3 (eg, codon 61)
81405	Molecular pathology procedure, Level 6 Gene: ***KRAS*** (Kirsten rat sarcoma viral oncogene homolog) (eg, Noonan syndrome), full gene sequence
88363	Examination and selection of retrieval archival (ie.: previously diagnosed) tissue(s) for molecular analysis (eg: *KRAS* mutational analysis)
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 Proprietary test: Praxis ™ Extended RAS Panel Lab/Manufacturer: Illumina

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

VIII. Evidence-based Scientific References

Allegra, C. J., Jessup, J. M., Somerfield, M. R., Hamilton, S. R., Hammond, E. H., Hayes, D. F., . . . Schilsky, R. L. (2009). American Society of Clinical Oncology provisional clinical opinion: testing for KRAS gene mutations in patients with metastatic colorectal carcinoma to predict response to anti-epidermal growth factor receptor monoclonal antibody therapy. *J Clin Oncol, 27*(12), 2091-2096. doi:10.1200/jco.2009.21.9170

Allegra, C. J., Rumble, R. B., Hamilton, S. R., Mangu, P. B., Roach, N., Hantel, A., & Schilsky, R. L. (2016). Extended RAS Gene Mutation Testing in Metastatic Colorectal Carcinoma to Predict Response to Anti-Epidermal Growth Factor Receptor Monoclonal Antibody Therapy: American Society of Clinical Oncology Provisional Clinical Opinion Update 2015. *J Clin Oncol, 34*(2), 179-185. doi:10.1200/jco.2015.63.9674

Argilés, G., Tabernero, J., Labianca, R., Hochhauser, D., Salazar, R., Iveson, T., . . . Arnold, D. (2020). Localised colon cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol, 31*(10), 1291-1305. doi:10.1016/j.annonc.2020.06.022

ASCO. Retrieved from https://www.asco.org/research-guidelines/quality-guidelines/guidelines/gastrointestinal-cancer#/9766

Cenaj, O., Ligon, A. H., Hornick, J. L., & Sholl, L. M. (2019). Detection of ERBB2 Amplification by Next-Generation Sequencing Predicts HER2 Expression in Colorectal Carcinoma. *Am J Clin Pathol, 152*(1), 97-108. doi:10.1093/ajcp/aqz031

Chang, X. N., Shang, F. M., Jiang, H. Y., Chen, C., Zhao, Z. Y., Deng, S. H., . . . Nie, X. (2021). Clinicopathological Features and Prognostic Value of KRAS/NRAS/BRAF Mutations in Colorectal Cancer Patients of Central China. *Curr Med Sci, 41*(1), 118-126. doi:10.1007/s11596-021-2326-1

Chiorean, E. G., Nandakumar, G., Fadelu, T., Temin, S., Alarcon-Rozas, A. E., Bejarano, S., . . . Chamberlin, M. D. (2020). Treatment of Patients With Late-Stage Colorectal Cancer: ASCO Resource-Stratified Guideline. *JCO Global Oncology*(6), 414-438. doi:10.1200/JGO.19.00367

Clark, J. W., & Grothey, A. (2021, April 14). Systemic chemotherapy for nonoperable metastatic colorectal cancer: Selecting the initial therapeutic approach. Retrieved from https://www.uptodate.com/contents/systemic-therapy-for-nonoperable-metastatic-colorectal-cancer-selecting-the-initial-therapeutic-approach

Compton, C. (2020). Pathology and prognostic determinants of colorectal cancer - UpToDate. In K. Tanabe (Ed.), *UpToDate*. Waltham. MA.

De Roock, W., Claes, B., Bernasconi, D., De Schutter, J., Biesmans, B., Fountzilas, G., . . . Tejpar, S. (2010). Effects of KRAS, BRAF, NRAS, and PIK3CA mutations on the efficacy of cetuximab plus chemotherapy in chemotherapy-refractory metastatic colorectal cancer: a retrospective consortium analysis. *Lancet Oncol, 11*(8), 753-762. doi:10.1016/s1470-2045(10)70130-3

Douillard, J. Y., Oliner, K. S., Siena, S., Tabernero, J., Burkes, R., Barugel, M., . . . Patterson, S. D. (2013). Panitumumab-FOLFOX4 treatment and RAS mutations in colorectal cancer. *N Engl J Med, 369*(11), 1023-1034. doi:10.1056/NEJMoa1305275

EGAPP. (2013). Recommendations from the EGAPP Working Group: can testing of tumor tissue for mutations in EGFR pathway downstream effector genes in patients with metastatic colorectal cancer improve health outcomes by guiding decisions regarding anti-EGFR therapy? *Genet Med, 15*(7), 517-527. doi:10.1038/gim.2012.184

El-Deiry, W. S., Vijayvergia, N., Xiu, J., Scicchitano, A., Lim, B., Yee, N. S., . . . Reddy, S. (2015). Molecular profiling of 6,892 colorectal cancer samples suggests different possible treatment options

specific to metastatic sites. *Cancer Biol Ther, 16*(12), 1726-1737. doi:10.1080/15384047.2015.1113356

Fan, J.-Z., Wang, G.-F., Cheng, X.-B., Dong, Z.-H., Chen, X., Deng, Y.-J., & Song, X. (2021). Relationship between mismatch repair protein, RAS, BRAF, PIK3CA gene expression and clinicopathological characteristics in elderly colorectal cancer patients. *World J Clin Cases, 9*(11), 2458-2468. doi:10.12998/wjcc.v9.i11.2458

FDA. (2009). Vectibix Package Insert. Retrieved from https://www.accessdata.fda.gov/drugsatfda_docs/label/2009/125147s080lbl.pdf

FDA. (2012). Erbitux Package Insert. Retrieved from https://www.accessdata.fda.gov/drugsatfda_docs/label/2012/125084s225lbl.pdf

FDA. (2014). SUMMARY OF SAFETY AND EFFECTIVENESS DATA (SSED). Retrieved from https://www.accessdata.fda.gov/cdrh_docs/pdf11/P110027B.pdf

FDA. (2015). SUMMARY OF SAFETY AND EFFECTIVENESS DATA (SSED). Retrieved from https://www.accessdata.fda.gov/cdrh_docs/pdf14/P140023B.pdf

FDA. (2017). SUMMARY OF SAFETY AND EFFECTIVENESS DATA (SSED). Retrieved from https://www.accessdata.fda.gov/cdrh_docs/pdf16/P160038B.pdf

Formica, V., Lucchetti, J., Doldo, E., Riondino, S., Morelli, C., Argirò, R., . . . Roselli, M. (2020). Clinical Utility of Plasma KRAS, NRAS and BRAF Mutational Analysis with Real Time PCR in Metastatic Colorectal Cancer Patients-The Importance of Tissue/Plasma Discordant Cases. *Journal of clinical medicine, 10*(1), 87. doi:10.3390/jcm10010087

Frucht, H., & Lucas, A. L. (2021, January 21). Molecular genetics of colorectal cancer. *UpToDate.* Retrieved from https://www.uptodate.com/contents/molecular-genetics-of-colorectal-cancer

Jones, J. C., Renfro, L. A., Al-Shamsi, H. O., Schrock, A. B., Rankin, A., Zhang, B. Y., . . . Grothey, A. (2017). (Non-V600) BRAF Mutations Define a Clinically Distinct Molecular Subtype of Metastatic Colorectal Cancer. *J Clin Oncol, 35*(23), 2624-2630. doi:10.1200/jco.2016.71.4394

NCCN. (2021, January 21). NCCN Clinical Practice Guidelines in Oncology; Colon Cancer v2.2021. Retrieved from https://www.nccn.org/professionals/physician_gls/pdf/colon.pdf

NICE. (2020). Colorectal cancer. Retrieved from https://www.nice.org.uk/guidance/ng151/chapter/Recommendations#molecular-biomarkers-to-guide-systemic-anti-cancer-therapy

Rebersek, M., Mesti, T., Boc, M., & Ocvirk, J. (2019). Molecular biomarkers and histological parameters impact on survival and response to first- line systemic therapy of metastatic colorectal cancer patients. *Radiol Oncol, 53*(1), 85-95. doi:10.2478/raon-2019-0013

Sartore-Bianchi, A., Amatu, A., Porcu, L., Ghezzi, S., Lonardi, S., Leone, F., . . . Siena, S. (2019). HER2 Positivity Predicts Unresponsiveness to EGFR-Targeted Treatment in Metastatic Colorectal Cancer. *Oncologist, 24*(10), 1395-1402. doi:10.1634/theoncologist.2018-0785

Sepulveda, A. R., Hamilton, S. R., Allegra, C. J., Grody, W., Cushman-Vokoun, A. M., Funkhouser, W. K., . . . Nowak, J. A. (2017). Molecular Biomarkers for the Evaluation of Colorectal Cancer: Guideline From the American Society for Clinical Pathology, College of American Pathologists, Association for Molecular Pathology, and American Society of Clinical Oncology. *J Mol Diagn, 19*(2), 187-225. doi:10.1016/j.jmoldx.2016.11.001

Therkildsen, C., Bergmann, T. K., Henrichsen-Schnack, T., Ladelund, S., & Nilbert, M. (2014). The predictive value of KRAS, NRAS, BRAF, PIK3CA and PTEN for anti-EGFR treatment in metastatic colorectal cancer: A systematic review and meta-analysis. doi:10.3109/0284186X.2014.895036

Van Cutsem, E., Cervantes, A., Adam, R., Sobrero, A., Van Krieken, J. H., Aderka, D., . . . Arnold, D. (2016). ESMO consensus guidelines for the management of patients with metastatic colorectal cancer. *Ann Oncol, 27*(8), 1386-1422. doi:10.1093/annonc/mdw235

Xu, Q., Xu, A. T., Zhu, M. M., Tong, J. L., Xu, X. T., & Ran, Z. H. (2013). Predictive and prognostic roles of BRAF mutation in patients with metastatic colorectal cancer treated with anti-epidermal growth factor receptor monoclonal antibodies: a meta-analysis. *J Dig Dis, 14*(8), 409-416. doi:10.1111/1751-2980.12063