Testing for Colorectal Cancer Management

Policy Number: AHS – M2026	**Prior Policy Name and Number:** *KRAS NRAS BRAF Mutation Analysis in Colorectal Cancer*
Initial Effective Date: June 01, 2023	**Current Effective Date:** February 01, 2025
Line(s) of Business: HMO; PPO; QUEST Integration; Medicare; FEP	**Precertification:** Refer to the GTM Utilization Review Matrix

I. Policy Description

Colorectal cancer (CRC) involves the accumulation of genetic and epigenetic modifications within pathways that regulate proliferation, apoptosis, and angiogenesis resulting in carcinoma of the colon and rectum (Bardhan & Liu, 2013). Tumors originate in adenomas or flat dysplasia and evolve into different morphologic patterns with invasion and expansion (Compton, 2023a).

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, 2023b; Sepulveda et al., 2017).

For guidance on microsatellite instability or tumor mutational burden testing in colorectal cancer, please refer to AHS-M2178- Microsatellite Instability and Tumor Mutational Burden Testing.

II. Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Specifications pertaining to Medicare and Medicaid can be found in the "Applicable State and Federal Regulations" section of this policy document.

1) For all individuals with metastatic colorectal cancer, *KRAS, NRAS*, and *BRAF* mutation genotyping of the primary or the metastatic tumor **MEETS COVERAGE CRITERIA**.
2) For individuals with metastatic colorectal cancer for whom tumor tissue testing did not identify a mutation in *KRAS, NRAS*, **or** *BRAF*, HER2 amplification testing **MEETS COVERAGE CRITERIA**.

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 an individual's illness.

3) For all other situations not described above, testing for *KRAS, NRAS*, and *BRAF* mutations **DOES NOT MEET COVERAGE CRITERIA**.
4) For all other situations and/or mutations not described above, genotyping of the colorectal cancer tumor **DOES NOT MEET COVERAGE CRITERIA.**
5) To determine the prognosis of stage II colon cancer following surgery, gene expression profiling **DOES NOT MEET COVERAGE CRITERIA.**

NOTES:
Note: For 2 or more gene tests being run on the same platform, please refer to AHS-R2162-Reimbursement Policy.

III. Table of Terminology

Term	Definition
ASCO	American Society of Clinical Oncology
ACS	American Cancer Society
BRAF	*B-Raf proto-oncogene*
CEA	Carcinoembryonic antigen
CLIA-1988	Clinical Laboratory Improvement Amendments of 1988
CNA	Copy number alteration
CRC	Colorectal cancer
ctDNA	Circulating tumor deoxyribonucleic acid
DFS	Disease free survival
dMMR	Deficient MMR
EGAPP	Evaluation of Genomic Applications in Practice and Prevention
EGFR	*Epidermal growth factor receptor*
ERBB2	*Erb-B2 Receptor Tyrosine Kinase 2*
ESCP	European Society of Coloproctology
ESMO	European Society for Medical Oncology
EWG	European Working Group
1F1CDx	Foundation One Cdx
FDA	Food and Drug Administration
FFPE	Formalin-fixed paraffin-embedded
FISH	Fluorescence in-situ hybridization
FOLFOX4	5-fluorouracil, leucovorin, and oxaliplatin
HER2	Human epidermal growth factor receptor 2
HR	Hazard ratios
ICI	Immune checkpoint inhibition
IHC	Immunohistochemistry
KRAS	*Kirsten rat sarcoma*
LDTs	Laboratory-developed tests

mAbs	Monoclonal antibodies
mCRC	Metastatic colorectal cancer
MMR	Mismatch repair
MMR-P	Mismatch repair proficient
MPFS	Median progression free survival
MSI	Microsatellite instability
NCCN	National Comprehensive Cancer Network
NGS	Next-generation sequencing
NICE	National Institute for Health and Care Excellence
NRAS	*Neuroblastoma rat sarcoma virus*
NSABP	National Surgical Adjuvant Breast and Bowel Project
OR	Odds ratio
ORR	Objective response rate
OS	Overall survival
PCO	Provisional clinical opinion
PCR	Polymerase chain reaction
PFS	Progression-free survival rate
PIK3CA	*Phosphatidylinositol-4,5-Bisphosphate 3-Kinase Catalytic Subunit Alpha*
PL	Plasma
PTEN	*Phosphatase and TENsin homolog deleted on chromosome 10*
QALY	Quality-adjusted life year
RAF	*Rapidly accelerated fibrosarcoma*
RAS	*Rat sarcoma virus*
RS	Recurrence score
T	Tissue
TMB	Tumor mutational burden
WT	Wild type

IV. Scientific Background

Colorectal cancer (CRC) is the third leading cause of cancer-related deaths in the United States following lung cancer. The American Cancer Society (ACS) estimates 106,180 new cases of colon cancer and 44,850 new cases of rectal cancer for 2022. Overall, there is about a four percent lifetime risk of developing colorectal cancer (ACS, 2023). Metastatic colorectal cancer (mCRC), which occurs in 22% of patients with colorectal cancer, has a significantly poorer prognosis than colorectal cancer that hasn't metastasized. The five-year survival is 14% in patients with distant metastases from CRC, as compared to 71% for all CRC patients (El-Deiry et al., 2015; Wang et al., 2020).

Approximately one-quarter of the patients with colon cancer present with stage II disease (Kopetz, 2008). The current National Comprehensive Cancer Network (NCCN) guidelines include adjuvant chemotherapy as a treatment option in this setting, particularly for high-risk stage II patients, as determined by clinical and pathological parameters (NCCN, 2024). Although some of the routinely used

parameters for estimating recurrence risk, such as T-stage and mismatch repair (MMR) status, are well established, they may not be reliable predictors of recurrence risk in this population (Gray et al., 2011; Gunderson et al., 2010; Harris et al., 2008; Ribic et al., 2003; Sargent et al., 2010; Venook et al., 2013).

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 et al., 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 & Sanoff, 2024). 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, 2023). 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 five percent of colorectal cancer cases are found to overexpress *HER2* (Clark & Sanoff, 2024).

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).

Proprietary Testing

Gene expression assays have been commercially produced to predict prognosis of colon cancer. The 12-gene Oncotype DX Colon Cancer Assay (Genomic Health, Inc., Redwood City, CA) is a reverse transcriptase polymerase chain reaction–based assay that provides a Recurrence Score (RS) result (O'Connell et al., 2010). This test assesses the activity level of 12 genes (7 cancer-related genes, 5 reference genes), and this gene expression is scored from 1-100. This test is intended for resected stage II, MMR-P or stage III A/B colon cancer. Low risk is a score under 30, moderate risk is 31-40, and higher risk is ≥41 (Oncotype, 2024a, 2024b).

The ColDx assay (Almac Diagnostics, Craigavon, Northern Ireland) uses microarray technology for assessing the gene expression of 634 genes to stratify patients into low and high recurrence risk groups (Almac Group, 2024). ColDx identified 73 high risk patients with a hazard ratio of 2.62 during cross validation. In an independent validation, the assay identified high-risk patients with a hazard ratio of 2.53 (Kennedy et al., 2011).

ColoPrint (Agendia, Amsterdam, The Netherlands) is a gene expression classifier that uses whole-genome expression data of 18 key genes to distinguish patients with low versus high risk of disease relapse. In a study using 206 fresh frozen tumor tissue samples from 188 patients with stage I through IV CRC, ColoPrint classified “60% of patients as low risk and 40% as high risk,” and was “superior to

American Society of Clinical Oncology criteria in assessing the risk of cancer recurrence without prescreening for microsatellite instability" (Salazar et al., 2011). In a study of 416 stage II colon cancer patients, "ColoPrint identified 63% of patients as low risk with a 5-year ROR of 10%, whereas high-risk patients (37%) had a 5-year ROR of 21%." Alternatively, the 2013 NCCN clinical risk factors could not distinguish low and high risk patients (Kopetz et al., 2015).

Analytical Validity

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 et al., 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.

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).

Pinheiro et al. (2022) studied the analytical validity of using ctDNA as a possible strategy to analyze *KRAS* and *NRAS* mutations from patients with metastatic colorectal cancer. The BEAMing Digital PCR (OncoBEAM) and Idylla ctDNA qPCR were compared and the concordance rate was reported. Blood samples from 47 mCRC patients were tested and the overall agreement and concordance rate were noted. "The overall agreement between tumor tissue and ctDNA analyses was 83% and 78.7% using the OncoBEAM and Idylla assays, respectively, with the concordance being 96.2% and 88.5% in naive treatment patients. The overall agreement between OncoBEAM and Idylla ctDNA analyses was 91.7%" (Pinheiro et al., 2022). The authors conclude that Idylla ctDNA qPCR method is a cheaper alternative with equivalent performance in comparison to the OncoBEAM assay. Analysis of ctDNA can be used to detect "RAS mutations in plasma, either at diagnosis or after progression when considering anti-EGFR treatment rechallenge" (Pinheiro et al., 2022).

Clinical Utility and Validity

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. A total of 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*). A total of 22 studies (2395 participants) 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. The study included 154 patients 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 et al., 2019).

Sartore-Bianchi et al. (2019) investigated the effect of *HER2* positivity on anti-*EGFR* treatment. A total of 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 seven 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).

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).

Loree et al. (2021) characterized the clinical prevalence of atypical *KRAS/NRAS* mutations in metastatic colorectal cancer. The authors evaluated tissue and DNA samples from 9,485 patients to characterize atypical *RAS* variants using an in-vitro cell-based assay, studying the signaling changes across mutations. According to the results, "*KRAS* exon 2, extended *RAS*, and atypical *RAS* mutations were noted in 37.8%, 9.5%, and 1.2% of patients, respectively. Among atypical variants, *KRAS* L19F, Q22K, and D33E occurred at prevalence ≥0.1%, whereas no *NRAS* codon 117/146 and only one *NRAS* codon 59 mutation was noted. Atypical *RAS* mutations had worse overall survival than *RAS/BRAF* wild-type mCRC.” Of the 57 atypical *RAS* variants, 18 (31.6%) had signaling below wild-type, 23 (40.4%) had signaling between wild-type and activating control, and 16 (28.1%) were hyperactive beyond the activating control. The authors concluded that "*KRAS* L19F, Q22K, D33E, and T50I are more prevalent than many guideline-included RAS variants and functionally relevant” (Loree et al., 2021).

Benavides et al. (2022) studied how effective liquid biopsy-tailored assays were in identifying guideline-recommended biomarkers, including *RAS* and *BRAF*, in comparison to standard of care tissue genotyping for patients newly diagnosed with mCRC. To quantify the effectivity of liquid biopsy assays for biomarkers, the researchers utilized the Guardant360 for comprehensive ctDNA analysis, and OncoBEAM for targeted RAS and BRAF analysis. Among the 155 patients included in this prospective study, physician discretion standard of care tissue genotyping identified guideline-recommended biomarkers in 52.9% of patients, in comparison to the 56.8% from the comprehensive Guardant360 ctDNA analysis and 44.5% from targeted ctDNA analysis by OncoBEAM. An additional 19.5% more samples were included in the ctDNA assays “by rescuing those without tissue results either due to tissue insufficiency, test failure, or false negatives.” The complete processing of ctDNA assays was faster (10 days versus 27 days on median) and maintained accuracy even 10 days after sample collection (52.0% vs 10.2%). This could allow inclusion of ctDNA genotyping in the care of patients with mCRC and could

enable accelerated personalized treatment regimens for patients with the quick turnaround and comparable results to current practices (Benavides et al., 2022).

Several studies have evaluated the impact of the gene expression profiling on clinical decision making in certain colon cancer subgroups. Brenner et al. (2016) assessed the clinical impact of the 12-gene Colon Cancer Recurrence Score Assay in treatment of T3 mismatch repair proficient (MMR-P) stage II colon cancer. Out of 269 patients, 102 patients had their treatment changed because of the assay's results. The authors concluded that testing significantly impacted adjuvant treatment decisions in clinical practice (Brenner et al., 2016).

Cartwright et al. (2014) performed a web-based survey evaluating the impact of the 12-gene Colon Cancer Recurrence Score Assay in stage II colon cancer patients. The authors surveyed 346 oncologists about their use of the Oncotype DX assay; the survey included questions about courses of treatment before and after using the assay and the stage of cancer their patient had. The authors found that 29% of treatment recommendations were changed for patients receiving Recurrence Score testing (Cartwright et al., 2014). Srivastava et al. (2014) conducted a prospective study assessing the impact of recurrence score results on physician recommendations regarding adjuvant chemotherapy in T3 MMR-P stage II colon cancer patients. A total of 141 patients were eligible for analysis, and the study concluded that treatment recommendation changes were made for 63 (45%) of patients (Srivastava et al., 2014).

Chang et al. (2020) reviewed the "entire database" of the OncoType Colon Recurrence Score test to identify any age-related differences in Recurrence Score (RS) and single-gene results. A total of 20478 Stage II and IIIA/B colon cancer patients were included. RS results were categorized into low, medium, and high risk, and single-gene results were organized by median and interquartile ranges. In total, 72.5% of all patients and 72.6% of patients under 40 years old were found to have a low-risk RS. However, there were no significant differences in either RS or single-gene results among the four age groups (<40, 40-54, 55-64, >65). Young-onset cancer was also not found to differ by gene expression in individual RS genes. Overall, most patients in stages II or III colon cancer were found to have low-risk disease per the OncoType assay (Chang et al., 2020).

Allar et al. (2022) evaluated how the OncoType Colon Recurrence Score influences clinical practice. The study included 105 patients with stage IIa colon cancer and investigated the association between the RS and the decision to offer adjuvant chemotherapy after resection. Fifty-two patients underwent RS testing, seven (13%) of whom received adjuvant chemotherapy. The authors found no significant effect or clear association of RS on the odds of undergoing chemotherapy. The authors conclude that "RS was not associated with the decision to start adjuvant chemotherapy" and suggest that "the RS should not be obtained in patients with stage IIa colon cancer" (Allar et al., 2022).

Chaudhari and Issa (2022) conducted a study to compare the cost-effectiveness of various genomic tests used to prognosticate stage II colorectal cancer patients. The researchers compared a 12-gene assay, 18-gene expression assay, 482-gene signature assay, and Immunoscore assay in a hypothetical cohort to investigate recurrence risk and death. Using a Markov model, the authors found that "the cost of the Immunoscore assay strategy in stage II colorectal cancer patients was estimated to be US $23,564 with a gain of 3.903 quality-adjusted life years (QALYs) as compared with the 12-gene assay strategy at US $24,545 and 3.903 QALYs; the 18-gene assay strategy at US $28,374 and 3.623 QALYs; and the 482-gene

signature treatment strategy at US $33,315 with 3.704 QALYs." This, along with further analysis, led to the conclusion that the Immunoscore assay may be the "dominant strategy," in that it may reduce costs associated with treatment in long-term, but for the gene expression signature assays alone, the 12-gene assay may generate more cost savings than the 18-gene expression assay, equivalent to $3900 (Chaudhari & Issa, 2022).

Aoki et al. (2023) studied the validity of NGS-based ctDNA genotyping for *RAS* and *BRAF* V600E mutation assessment to guide therapy for metastatic colorectal cancer. The study included 212 mCRC patients. The authors compared NGS-cased ctDNA genotyping results with the results of validated PCR-based tissue testing, specifically looking at the concordance rate, sensitivity, and specificity. For *RAS,* the concordance rate was 92.5%, the sensitivity was 88.7%, and the specificity was 97.2%. For *BRAF* V600E, the concordance rate was 96.2%, the sensitivity was 88.0%, and the specificity was 97.3%. The authors then investigated efficacy of anti-EGFR and BRAF-targeted therapies based on ctDNA results. The progression-free survival pf anti-EGFT therapy was 12.9 months, and the progression-free survival of BRAF-targeted treatment was 3.7 months. The authors concluded that "ctDNA genotyping effectively detected RAS/BRAF mutations" and "clinical outcomes support ctDNA genotyping for determining the use of anti-EGFR and BRAF-targeted therapies in patients with mCRC" (Aoki et al., 2023).

V. Guidelines and Recommendations

American Society of Clinical Oncology (ASCO)

The ASCO published an endorsement of the College of American Pathologist Guidelines, recommending:

- "For patients with CRC, being considered for immune checkpoint inhibitor therapy, pathologists should use MMR-immunohistochemistry (IHC) and/or microsatellite instability (MSI) by polymerase chain reaction (PCR) for the detection of DNA MMR defects. Although MMR-IHC or MSI by PCR is preferred, pathologists may use a validated MSI by next-generation sequencing (NGS) assay for the detection of DNA MMR defects. Note: MSI by NGS assay must be validated against MMR-IHC or MSI by PCR and must show equivalency. (Strong recommendation)."

- "For all cancer patients being considered for immune checkpoint inhibitor therapy based on defective MMR, pathologists should not use tumor mutation burden (TMB) as a surrogate for the detection of DNA MMR defects. If a tumor is identified as TMB-high, pathologists may perform IHC and/or MSI by PCR to determine if high TMB is secondary to MMR deficiency. (Strong recommendation)."

- "For cancer patients being considered for immune checkpoint inhibitor therapy, if a MMR deficiency consistent with Lynch syndrome is identified in the tumor, pathologists should communicate this finding to the treating physician. (Strong recommendation)" (Vikas et al., 2023).

American Society for Clinical Pathology, College of American Pathologists, Association for Molecular Pathology, and the American Society of Clinical Oncology

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:

- "Patients with CRC 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 CRC tissue in patients with CRC 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).

The joint guidelines state that further research is required to study the clinical validity and utility of gene expression profiling assays in colon cancer patients (Sepulveda et al., 2017).

National Comprehensive Cancer Network (NCCN)

The guidelines version 1.2024 recommend that "all patients with metastatic colorectal cancer should have tumor genotyped for *RAS* (*KRAS* and *NRAS*) and *BRAF* mutations individually or as part of a next-generation sequencing (NGS) panel (preferred). Patients with any known *KRAS* mutation (exons 2, 3, and 4) or *NRAS* mutation (exons 2, 3, and 4) should not be treated with either cetuximab or panitumumab, unless given as part of a regimen targeting a *KRAS* G12C mutation. *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." Testing can be performed on formalin fixed paraffin embedded tissue (preferred) or blood-based assay.

The NCCN further states that "testing can be performed on the primary CRCs 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 despite being rarely amplified/overexpressed in CRC (~3% overall). HER2-targeted therapies are now recommended in patients with tumors that are *RAS/BRAF* wild-type and with HER2 overexpression. Therefore, the NCCN now recommends testing for HER2 amplifications in patients with metastatic CRC. However, *HER2* testing is not indicated in patients with known *KRAS*/*NRAS* or *BRAF* mutations (NCCN, 2024).

Routine *EGFR* testing is not recommended (NCCN, 2024).

Overall, in patients with suspected or proven mCRC, the NCCN recommends "molecular testing, including: *RAS* and *BRAF* mutations; HER2 amplifications; MMR or MSI status (if not previously done). Testing should be conducted as part of broad molecular profiling, which would identify rare and actionable mutations and fusions such as *POLE/POLD1, RET,* and *NTRK*." (NCCN, 2024).

Regarding the OncoType DX colon cancer assay, the NCCN remarks that clinical validation in patients with stages II or III cancer from the QUASAR and NSABP clinical trials shows that "recurrence scores are prognostic for recurrence, DFS [disease free survival], and OS [overall survival] in stage II and stage III colon cancer but are not predictive of benefit to adjuvant therapy." ColoPrint, an 18-gene classifier for recurrence risk, was also found to independently predict recurrence risk and is currently being validated to predict 3-year relapse rates in patients with stage II colon cancer in a prospective trial. Similarly, ColDx, a microarray based multigene assay, was found to independently predict recurrence risk. However, despite these tests' ability to further inform risk of recurrence, the panel questions the value added. The panel also noted that "evidence of predictive value in terms of the potential benefit of chemotherapy is lacking" and that "there are insufficient data to recommend the use of multigene assays, Immunoscore, or post-surgical ctDNA to estimate risk of recurrence or determine adjuvant therapy" (NCCN, 2024).

European Society for Medical Oncology (ESMO)

In its 2023 guidelines, ESMO recommends the following for mCRC genetic testing:

- "Determining the *RAS* mutational testing on a tumour biopsy [I, A] (or through a liquid biopsy in case no tumour sample is available [II, B]) is mandatory to guide the best treatment decision.
- Testing for mismatch repair (MMR) status and *KRAS, NRAS* exon 2, 3, and 4 as well as *BRAF* mutations is recommended in all patients at the time of mCRC diagnosis [I, A]
- Identification of human epidermal growth factor receptor (HER2) amplification by immunohistochemistry (IHC) or FISH [fluorescence in-situ hybridization] is recommended in *RAS* wild-type (wt) patients to detect those who may benefit from HER2 blockade [III, B]
- *RAS* testing is mandatory before treatment with anti-EGFR mAbs and can be carried out on either the primary tumour or other metastatic sites [III, A]
- *BRAF* mutation status should be assessed simultaneously with the evaluation of *RAS*, for prognostic assessment [I, B] and for the option of treatment with cetuximab-encorafenib [I, A].
- dMMR [deficient mismatch repair]/MSI testing in mCRC can assist in genetic counselling for Lynch syndrome [II, B].
- dMMR/MSI status is also recommended as the initial molecular work-up in metastatic disease for its predictive value for the use of ICIs [immune checkpoint inhibition] [I, A]" (Cervantes et al., 2023).

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).

In their newly released guidelines, ESMO does not provide recommendations for using gene expression profiling assays for prognosticating patients with stage II colon cancer (Cervantes et al., 2023).

Choosing Wisely Canada

Choosing Wisely Canada lists "eleven tests and treatments to question" in their oncology recommendations. In this list, they recommend: "Don't perform routine colonoscopic surveillance every year in patients following their colon cancer surgery; instead, frequency should be based on the findings of the prior colonoscopy and corresponding guidelines" (Choosing Wisely Canada, 2023).

Research Committee and the Guidelines Committee of the European Society of Coloproctology (ESCP)

This systematic review was performed by the committee to assess the consensus levels "in guidelines from member countries of the European Society of Coloproctology, with supporting evidence." This review focuses on follow-up strategies for patients "after treatment with curative intent of nonmetastatic colorectal cancer" (Bastiaenen et al., 2019).

In this review, the committee concluded that "laboratory tests other than CEA [carcinoembryonic antigen] should not be part of follow-up," although it noted that only eight of 21 guidelines reviewed addressed this topic (Bastiaenen et al., 2019).

VI. Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website. For the most up-to-date Medicaid policies and coverage, visit the applicable state Medicaid website.

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).

In 2021, the ONCO/Reveal Dx Lung & Colon Cancer Assay (O/RDx-LCCA) was approved. O/RDx-LCCA is a highly accurate FDA approved IVD assay for the detection of clinically relevant KRAS variants in CRC and EGFR variants in NSCLC and determination of approved therapy. "The device is a qualitative next generation sequencing based in vitro diagnostic test that uses amplicon-based target enrichment technology for detection of single nucleotide variants (SNVs) and deletions in 2 genes from DNA isolated from formalin-fixed paraffin-embedded (FFPE) non-small cell lung cancer (NSCLC) and colorectal cancer (CRC) tumor tissue specimens. The test is intended as a companion diagnostic to identify patients with NSCLC or CRC who may benefit from treatment with the targeted therapies" (FDA, 2021).

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). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

VII. Important Reminder

The purpose of this Medical Policy is to provide a guide to coverage. This Medical Policy is not intended to dictate to providers how to practice medicine. Nothing in this Medical Policy is intended to discourage or prohibit providing other medical advice or treatment deemed appropriate by the treating physician.

Benefit determinations are subject to applicable member contract language. To the extent there are any conflicts between these guidelines and the contract language, the contract language will control.

This Medical Policy has been developed through consideration of the medical necessity criteria under Hawaii's Patients' Bill of Rights and Responsibilities Act (Hawaii Revised Statutes §432E-1.4) or for QUEST members, under Hawaii Administrative Rules (HAR 1700.1-42), generally accepted standards of medical practice and review of medical literature and government approval status.

HMSA has determined that services not covered under this Medical Policy will not be medically necessary under Hawaii law in most cases. If a treating physician disagrees with HMSA's determination as to medical necessity in a given case, the physician may request that HMSA reconsider the application of the medical necessity criteria to the case at issue in light of any supporting documentation.

Genetic testing is covered for level 1 or 2A recommendations of the National Comprehensive Cancer Network (NCCN and in accordance with Hawaii's Patients' Bill of Rights and Responsibilities Act (Hawaii Revised Statutes §432E-1.4) or for QUEST members, the Hawaii Administrative Rules (HAR 1700.1-42).

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IX. Policy History

Action Date	Action
June 01, 2023	Policy created
December 03, 2024	Policy approved by Medical Directors
December 20, 2024	Policy approved at UMC
February 01, 2025	Policy effective date following notification period