Clinical UM Guideline

Subject:	Analysis of RAS Status		
Guideline #:	CG-GENE-02	**Publish Date:**	09/25/2019
Status:	Revised	**Last Review Date:**	08/22/2019

Description

This document addresses DNA testing used to determine the status of the RAS gene family (KRAS and NRAS) used to guide therapy decisions in individuals with oncologic conditions. This document does not address the status of the HRAS gene.

For additional information, please refer to the following related documents:

- CG-GENE-03 BRAF Mutation Analysis
- CG-GENE-12 PIK3CA Mutation Testing for Malignant Conditions
- CG-GENE-13 Genetic Testing for Inherited Diseases
- CG-GENE-20 Epidermal Growth Factor Receptor (EGFR) Testing
- GENE.00010 Genotype Testing for Genetic Polymorphisms to Determine Drug-Metabolizer Status
- GENE.00038 Genetic Testing for Statin-Induced Myopathy

Clinical Indications

Medically Necessary:

Analysis of RAS (KRAS and NRAS) status is considered **medically necessary** as a technique to predict treatment response to the anti-EGFR monoclonal antibody cetuximab (Erbitux), or panitumumab (Vectibix) in individuals with stage IV colon, rectal, colorectal or anal adenocarcinoma prior to initiation of cetuximab or panitumumab.

Not Medically Necessary:

The analysis of RAS status (KRAS and NRAS) is considered **not medically necessary** for all applications not indicated above as medically necessary including but not limited to routine testing at initial diagnosis.

Coding

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

CPT	
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)
88363	Examination and selection of retrieved archival (ie, previously diagnosed) tissue(s) for molecular analysis (eg, KRAS mutational analysis) [when specified in relation to KRAS or NRAS testing]
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 Praxis™ Extended RAS Panel, Illumina, Illumina

ICD-10 Diagnosis	
C18.0-C18.9	Malignant neoplasm of colon
C19	Malignant neoplasm of rectosigmoid junction
C20	Malignant neoplasm of rectum
C21.0-C21.8	Malignant neoplasm of colon, rectum, rectosigmoid junction, anus
C78.5	Secondary malignant neoplasm of large intestine and rectum

Discussion/General Information

Colorectal Cancer

RAS gene mutation status is being evaluated as a possible biomarker of individuals with metastatic (Stage IV) colorectal cancer (mCRC) who may not respond well to anti-EGFR monoclonal antibody (mAb) drugs. Because there are few treatment options for individuals with metastatic colorectal cancer, the most likely clinical application of RAS status analysis would be to identify those individuals who would not respond to anti-EGFR mAb therapy, thereby saving them the time, expense and unnecessary toxicity of ineffective therapies. Both cetuximab (Erbitux) and panitumumab (Vectibix) are approved by the U.S. Food and Drug Administration (FDA) as anti-EGFR agents for the treatment of metastatic colorectal cancer in individuals with refractory disease. Cetuximab is specifically indicated in KRAS wild-type mCRC, and panitumumab is specifically indicated in RAS wild-type (defined as wild-type in both KRAS and NRAS) mCRC.*

***Note:** RAS wild-type means the gene is normal or lacking mutations.*

Cervantes and colleagues (2008) assessed the efficacy of cetuximab alone and in combination with chemotherapy in the first-line setting. The researchers analyzed the KRAS status in 48 individuals with EGFR-expressing metastatic CRC who received weekly versus bi-weekly administration of cetuximab. Initially, cetuximab was administered as a first-line single therapeutic agent for 6 weeks, then individuals were assessed for a response to therapy, and lastly, FOLFIRI (leucovorin [folinic acid], 5-FU [flurorouracil] and irinotecan) chemotherapy was

added to all participants. KRAS status was determined using archived tumor material. Histopathologic evaluation revealed that 48 of the 52 samples contained tumor tissue. All 48 individuals were evaluated for efficacy. KRAS mutations were detected in 19 samples (40%). At the conclusion of the monotherapy phase, the response rate was 27.6% for the KRAS wild type group and 0% for the KRAS mutant group (p=0.015). During the combination phase, this response rate rose to 55.2% vs. 31.6%, respectively (p=0.144). Progression free survival for cetuximab in combination with FOLFIRI was significantly improved (hazard ratio[HR]: 0.47, p=0.0475) for KRAS wild type group compared to KRAS mutant type group. The authors concluded that KRAS mutation status can be used to determine the efficacy of cetuximab in the first-line setting for individuals with metastatic CRC either alone or when subsequently used in combination with FOLFIRI.

Van Cutsem and colleagues (2008) conducted efficacy analyses to evaluate the effect of KRAS status in individuals receiving first-line treatment with FOLFIRI with or without cetuximab under controlled study conditions. Building upon the results of the CRYSTAL trial in which 1198 individuals with metastatic CRC were randomized to receive either cetuximab in combination with FOLFIRI, 5-FU and irinotecan or FOLFIRI alone, a subgroup analysis assessing the response rate and progression free survival based on the KRAS status was carried out. Of the 1198 original study participants, 540 individuals had KRAS-evaluable achievable tumor material. KRAS mutations were found in 192 (35.6%) of the individuals. Participants with wild type KRAS status who received cetuximab in combination with FOLFIRI exhibited a statistically significant improvement in progression free survival, as compared with those with the KRAS mutation. There was no significant difference in the mutant type KRAS individuals on FOLFIRI alone when compared to the group that received cetuximab in combination with FOLFIRI. The researchers concluded that this study demonstrates the predictive value of KRAS status for the treatment of cetuximab in combination with FOLFIRI as a first-line treatment of metastatic CRC. Individuals with the KRAS mutant type did not demonstrate a benefit from treatment with cetuximab.

The OPUS study was a randomized controlled trial that compared FOLFOX (leucovorin [folinic acid], 5-FU [flurorouracil] and oxaliplatin) alone to FOLFOX in combination with cetuximab for first-line treatment of colorectal cancer. The OPUS trial had previously shown an improved response rate when cetuximab was used in combination with FOLFOX, but no improvement was noted in progression-free survival. Bokemeyer and colleagues (2008) repeated the efficacy analyses of the OPUS trial to evaluate KRAS status and the influence of first-line treatment of individuals with metastatic CRC with FOLFOX with or without cetuximab. KRAS mutations were detected in 99 of 233 (42%) of the evaluable samples. The data suggest that the population with wild-type KRAS status benefited more (improved response rate and progression free survival) from the addition of cetuximab to standard treatment, as compared with those with the KRAS mutation.

A published study (Karapetis, 2008) analyzed KRAS status in tumor samples obtained from 394 of 572 individuals with colorectal cancer who had been randomly assigned to receive either cetuximab plus best supportive care, or best supportive care alone. Cetuximab effectiveness was significantly associated with KRAS status; in those with the wild-type version, adding cetuximab to best supportive care alone improved median overall survival (9.5 vs. 4.8 months), HR for death (0.55), and median progression-free survival (3.7 vs. 1.9 months). Among individuals with mutant KRAS, there was no benefit offered by adding cetuximab to best supportive care. In all individuals receiving best supportive care alone without cetuximab, KRAS status was not significantly associated with

improved overall survival (HR for death 1.01). An accompanying editorial (Messersmith, 2008) stated it is reasonable to recommend that all individuals with advanced colorectal cancer being considered for anti-EGFR therapy should undergo KRAS testing, and if the mutant gene is detected, anti-EGFR therapy should not be administered.

De Roock and colleagues (2008) conducted a retrospective analysis of 113 individuals with irinotecan-refractory metastatic colorectal carcinoma with available tumor tissue, from four Belgian clinical trials. A total of 102 individuals had completed tumor measurements throughout the clinical trials. The clinical trials utilized cetuximab as monotherapy and in combination therapy. KRAS mutations were detected in 46 of 113 (40.7%) tumors and BRAF V600E mutation was noted in 6 of 107 (5.6%) assessable participants. The combined BRAF and KRAS mutations were not identified in any individual. Five individuals were not assessable prior to the first evaluation. Overall response (OR), including both complete (CR) and partial response (PR), was observed only in the individuals with KRAS wild-type (27 of 66 [41%]) compared to individuals with KRAS mutants (0 of 42 [0%]). KRAS mutations were identified in 42 of 81 (51.9%) nonresponders, and in 27 OR individuals. There was no statistically significant difference in the median progression-free survival (PFS) in the entire study. However, in the cohort receiving cetuximab in combination therapy, there was a significant median PFS between wild-type KRAS (34 weeks) compared to mutant KRAS (12 weeks; p=0.016). In the entire study population, there was also a significant difference in median overall survival (OS) for KRAS wild-type 43 weeks compared to KRAS mutants 27.3 weeks (p=0.020). The authors concluded KRAS wild-type is a strong predictor of a significant increase in PFS and OS. However, the KRAS status of a tumor may still fall short as a biomarker, as not all individuals with wild-type respond or have improved survival and some individuals with mutant KRAS experience long-term disease control. The data suggest that the metastatic colorectal population with wild-type KRAS mutation status benefited more from cetuximab as compared with those with the activating KRAS mutation. Therefore, analysis of KRAS may be appropriate to facilitate treatment plans.

In a study of using panitumumab versus best supportive care in a large series of such individuals, Amado and colleagues (2008) found that response rates to the drug were significantly better in those with wild-type KRAS status as compared with those with the mutant variety (response rates 17% vs. 0%, and longer overall survival). The authors concluded that KRAS status should be considered when selecting such individuals for panitumumab monotherapy.

Bokemeyer and colleagues (2012) conducted a pooled analysis to further evaluate the findings of the CRYSTAL (Cetuximab Combined with Irinotecan in First-Line Therapy for Metastatic Colorectal Cancer) trial and the OPUS (Oxaliplatin and Cetuximab in First-Line Treatment of mCRC) study using extended survival data. In participants evaluable for KRAS and BRAF mutation status, pooled individual subject data from each study were analyzed for overall survival (OS), progression-free survival (PFS) and best overall response rate (ORR). The treatment arms were compared according to mutation status using log-rank and Cochran-Mantel-Haenszel tests. Of the 845 subjects with KRAS wild-type tumors, the addition of cetuximab to chemotherapy led to a significant improvement in OS (HR 0.81; p=0.0062), PFS (HR 0.66; p<0.001) and ORR (odds ratio 2.16; p<0.0001). The authors concluded that the findings confirm the consistency of the benefit obtained across all efficacy end-points from adding cetuximab to first-line chemotherapy in individuals with KRAS wild-type metastatic CRC.

In 2015, Sorich and colleagues published the results of a systematic review and meta-analysis of randomized controlled trials that evaluated extended RAS mutations and anti-EGFR mAb survival benefit in mCRC. Specifically, the authors evaluated trials with the following inclusion criteria: KRAS mutations in exon 3 (codon 59, 61) or exon 4 (codons 117, 146), or NRAS mutations in exon 2, 3 or 4 and follow-up for PFS or OS. The systematic review yielded 9 randomized controlled trials with a total of 5948 participants. Cetuximab versus no cetuximab was studied in 3 trials (overall intention-to-treat=3165), panitumumab versus no panitumumab was studied in 4 trials (overall intention-to-treat=3447), cetuximab versus bevacizumab was studied in 1 trial (overall intention-to-treat=735), and panitumumab versus bevacizumab was studied in 1 trial (overall intention-to-treat=285). The results of the meta-analysis showed:

> Approximately 20% of KRAS exon 2 wild-type tumors harbored one of the new RAS mutations. Tumors without any RAS mutations (either KRAS exon 2 or new RAS mutations) were found to have significantly superior anti-EGFR mAb PFS ($p<0.001$) and OS ($p=0.008$) treatment effect compared with tumors with any of the new RAS mutations. No difference in PFS or OS benefit was evident between tumors with KRAS exon 2 mutations and tumors with the new RAS mutations. Results were consistent between different anti-EGFR agents, lines of therapy and chemotherapy partners. Anti-EGFR mAb therapy significantly improved both PFS (HR 0.62 [95% confidence interval (CI) 0.50–0.76]) and OS (HR 0.87 [95% CI 0.77–0.99]) for tumors without any RAS mutations. No PFS or OS benefit was evident with use of anti-EGFR mAbs for tumors harboring any RAS mutation ($p>0.05$).

A limitation to note is that some of the studies included were presented at conferences and were not published, thus, did not fully go through the peer-review process. This systematic review and meta-analysis further supports that testing of wild type for all known KRAS and NRAS activating mutations in mCRC individuals is necessary prior to cetuximab or panitumumab treatment.

The guidelines published by the European Society of Medical Oncology (ESMO) support the determination of RAS status (KRAS and NRAS) as a prerequisite to anti-EGFR treatment for metastatic CRC (Van Cutsem, 2014; Van Cutsem, 2016).

The 2015 American Society of Clinical Oncology (ASCO) Provisional Consensus Opinion addresses recent evidence suggesting that RAS mutations of both KRAS and NRAS may be predictive of resistance to treatment with monoclonal antibodies (currently panitumumab and cetuximab) targeting the EGFR. ASCO recommends that:

> All patients with mCRC 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 (Allegra, 2015).

The 2017 American Society for Clinical Pathology, College of American Pathologists, Association for Molecular Pathology, and American Society of Clinical Oncology guideline entitled Molecular Biomarkers for the Evaluation of Colorectal Cancer includes the recommendation that:

> Patients with colorectal carcinoma being considered for anti-EGFR therapy must receive RAS mutational testing. 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 ("expanded" or "extended" RAS) (Sepulveda, 2017).

The updated National Comprehensive Cancer Network (NCCN) guidelines on Colon Cancer (V1.2019) and the guidelines on Rectal Cancer (V2.2019) include recommendations for KRAS and NRAS gene testing for metastatic colon and rectal disease. Use of cetuximab or panitumumab is indicated for individuals with tumors that express the wild-type RAS gene. The NCCN also recommends that individuals with anal adenocarcinoma be managed according to their Rectal Cancer guidelines.

In summary, clinical studies and guidelines suggest that those individuals with metastatic colorectal tumors demonstrating wild-type RAS status (KRAS and NRAS) are more likely to benefit from cetuximab or panitumumab than those individuals whose tumors demonstrate a RAS mutation (KRAS and NRAS). Therefore, analysis of RAS (KRAS and NRAS) may be appropriate to facilitate treatment plans for individuals with metastatic colorectal tumors, both in terms of enhancing efficacy and preventing unnecessary adverse effects.

Non-small-cell Lung Cancer
RAS gene mutation status is also being evaluated as a tool to better manage individuals with other types of cancer including, but not necessarily limited to non-small-cell lung cancer (NSCLC), esophageal, pancreatic, gastric and endometrial cancer. However, research demonstrating the efficacy of RAS status in influencing clinical outcomes has not proceeded as rapidly for these indications as it has for metastatic colorectal cancer.

While there are some studies suggesting that KRAS status testing may be useful in select individuals with NSCLC, no general consensus has been reached regarding its role in improving clinical management or individual outcomes. Several randomized controlled trials suggest that additional research is needed in order to determine how KRAS status can be used to determine which tumors are likely to respond to select pharmacologic agents and the impact of KRAS status on the survival outcomes of individuals with NSCLC (Douillard, 2010; Eberhard, 2005; Khambata-Ford, 2010; Schneider, 2008). Mao and colleagues (2010) carried out a meta-analysis that included 22 studies. The final analysis consisted of a total of 1470 individuals with NSCLC of whom 231 (16%) had KRAS mutations. The objective response rates for individual with a KRAS mutation was 3% (6/210), as opposed to the response rate in individuals with the wild-type KRAS which was 26% (287/1125). Subgroup analyses were carried out on the basis of ethnicity and study treatment; however, the results were not materially altered. The researchers concluded that KRAS mutations may represent negative predictive biomarkers for tumor response in individuals with NSCLC treated with EGFR-tyrosine kinase inhibitors (TKIs). However, due to the mutually exclusive relationship between KRAS and EGFR mutation and no difference in survival between KRAS mutant/EGFR wild-type and KRAS wild-

type/EGFR wild-type NSCLC, the clinical usefulness of KRAS mutation as a selection marker for EGFR-TKIs sensitivity in NSCLC is limited."

Although current NCCN guidelines on NSCLC (V5.2019) indicate that KRAS mutational status is prognostic of survival and is predictive of therapeutic efficacy from EGFR-TKIs, specific recommendations are not made with regard to the use of KRAS mutation testing in the selection of TKI and anti-EGFR mAb therapies for NSCLC.

The updated evidence-based guidelines from the College of American Pathologists, the International Association for the Study of Lung Cancer, and the Association for Molecular Pathology addressing molecular testing to select individuals with lung cancer for treatment with targeted TKI inhibitors, which was endorsed by the ASCO (Kalemkerian, 2018), indicates that:

> KRAS molecular testing is not indicated as a routine stand-alone assay as a sole determinant of targeted therapy. It is appropriate to include KRAS as part of larger testing panels performed either initially or when routine EGFR, ALK, and ROS1 testing are negative (Lindeman, 2018).

Esophageal Cancer
Published studies evaluating the role of RAS in individuals with esophageal cancer are also limited. Working on the premise that KRAS status is a predictor of resistance to cetuximab therapy in colorectal cancer, researchers conducted a retrospective analysis of tumor samples from individuals with metastatic esophageal squamous cell carcinoma (ESCC) who were treated within the OESOTUX trial, to evaluate the KRAS status (Lorenzen, 2009). Chemo-naïve participants were randomized to receive either cetuximab plus cisplatin/5FU (CET-CF) or cisplatin/5FU (CF) alone. Tumor samples were collected from previous diagnostic or surgical procedures and were examined for the presence of somatic mutations in the KRAS gene. A total of 62 individuals with metastatic ESCC were included; of which 32 participants were allocated to the CET-CF and 30 of the participants to the CF arm. The individuals who were treated with CET-CF compared to individuals treated with CF alone tended to have a higher confirmed overall response rate (PR+CR) (19% versus 13%), a better disease control rate (CR+PR+SD) (75% versus 57%) as well as a longer median progression-free survival (5.9 [3.8-8.0] versus 3.6 [1.0-6.2] months) and a longer median overall survival (9.5 [8.4-10.6] and 5.5 [1.9-9.1] months). Tumor samples from 37 of the 62 individuals were analyzed for KRAS status. Researchers were unable to obtain tumor samples or deemed the samples inadequate for testing in 25 of the participants. No mutations of the KRAS gene could be detected in any of the 37 tumor samples that were evaluated. The authors concluded that:

> KRAS gene mutations in codon 12 and 13 are rare to absent events in ESCC. However, due to the small samples size, further studies are needed to confirm the findings of this subset analysis. Other activating mutations in the EGFR downstream signaling pathway cannot be excluded.

Pancreatic Cancer
The role of RAS is also being evaluated in individuals with pancreatic cancer or pancreatic cysts. Mucinous cysts which include intraductal papillary mucinous neoplasms (IPMNs) and mucinous cystic neoplasms (MCNs) have malignant potential. Current recommendations support the surgical removal of all symptomatic cysts and main

duct IPMNS provided the individuals are surgically fit. Benign cysts (serous cystic tumors), pseudocysts and retention cysts are not malignant; therefore management of such cysts does not include surveillance or resection. Branch duct IPMNs, which are slow to develop but do have malignant potential, are typically managed with resection and surveillance. Making the preoperative distinction between mucinous and nonmucinous cysts is not always clear. Endoscopic ultrasound (EUS) with fine-needle aspiration (FNA) is routinely used to evaluate pancreatic cysts. Carcinoembryonic antigen (CEA) is currently the most accurate marker of mucinous cysts. Cytologic evaluation of cyst fluid remains inaccurate for the evaluation of mucinous cysts and may not yield sufficient cellular material for diagnosis in the absence of a solid component. Cyst fluid DNA analysis is being explored as a tool in the identification of mucinous cysts (Rockacy, 2013).

At the time of this review, there were no published peer-reviewed randomized controlled trials evaluating the possible role of RAS status in individuals with pancreatic cancer. However, the results of at least one phase I clinical trial exploring the role of KRAS in individuals with pancreatic cancer has been published. In the study by Olson and colleagues (2009), researchers evaluated plasma KRAS as a potential marker of response to gefitinib and concurrent chemoradiation in 12 individuals with advanced pancreatic cancer. The researchers concluded changes in serum KRAS may provide critical information as to the efficacy of gefitinib and assist in tailoring treatment for cancers of the pancreas. Khalid and colleagues (2005) evaluated the role of molecular markers in mucinous cystic lesions of the pancreas. During a 19 month period, endoscopic ultrasound-guided pancreatic cyst aspirates were collected and studied for cytology, CEA level, and molecular analysis. Molecular evaluation incorporated DNA quantification (amount and quality), KRAS point mutation, and allelic loss analysis. Of the 36 cysts analyzed, 11 were malignant, 15 were premalignant, and 10 were benign. Malignant cysts were differentiated from premalignant cysts on the basis of fluid CEA level ($p=0.034$), DNA quality ($p=0.009$), number of mutations ($p=0.002$), and on the sequence of mutations acquired ($p<0.001$). Early KRAS mutation followed by allelic loss was the most predictive of a malignant cyst (sensitivity, 91%; specificity, 93%). The authors concluded that an initial KRAS mutation followed by allelic loss was the most predictive marker for carcinoma in a pancreatic cyst. Despite these preliminary findings, the authors concluded that the results needed to be validated in a larger series and outside of the research setting.

In a subsequent study (the PANDA [Pancreatic Cyst DNA Analysis] trial), Khalid and colleagues (2009) evaluated the utility of a detailed DNA analysis of pancreatic cyst fluid to diagnose nonmucinous (benign) and mucinous (malignant) cysts. The study consisted of 113 individuals with pancreatic cysts who underwent diagnostic aspiration cytology or surgical resection. The cysts were classified as nonmucinous (benign) or mucinous, with the mucinous group being further subdivided into pre-malignant and malignant (carcinoma in situ or invasive adenocarcinoma, respectively). Cyst fluid obtained by endoscopic ultrasound (EUS) and a detailed DNA analysis which included KRAS mutation analysis was used to establish the overall fraction of alleles deemed lost as compared to the germ line (mean allelic loss amplitude [MALA]). The presence of KRAS mutation or an MALA greater than 65% indicated a mucinous lesion by both a univariate and multivariate analysis and DNA analysis enhanced the sensitivity of cyst fluid CEA. KRAS mutation alone was not predictive of malignancy in mucin-producing cysts. A KRAS mutation in cyst fluid had a sensitivity of 45% for diagnosing a mucinous pancreatic cyst, with a specificity of 96%. The most accurate test for the diagnosis of a malignant cyst is an allelic loss amplitude (ALA) greater than 80% (sensitivity 70% and specificity 85%). The combination of KRAS and high

MALA as well as an MALA greater than 82% was associated with high-grade cysts that produced mucin. The authors concluded that a combination of tests which include cytologic evaluation, CEA level and a detailed DNA analysis can improve the diagnostic yield of pancreatic cyst fine needle aspiration (FNA). The authors recommend that if a cyst has a solid component, it should be specifically targeted for cytologic evaluation. If the cyst cytologic examination is negative for malignancy, a detailed DNA analysis would be helpful. The authors also concluded that the presence of a KRAS mutation is almost diagnostic for a mucinous cyst and in its absence, the CEA level should be used to differentiate between mucinous and nonmucinous cysts. The authors acknowledged several shortcomings of the study, including a selection bias. The evaluators included only 113 of the 299 participants and only those who underwent EUS-guided FNA and had either malignant cytology or definitive surgical pathology. By excluding the other 186 participants, a selection bias was introduced which potentially overestimated the performance of the DNA analysis and restricted generalization of the study results. Although current recommendations for the surgical management of mucinous cysts are based upon patient symptoms, EUS findings or other imaging results which typically include the size, location, appearance and patient preference, the clinical decision process used by the evaluators to determine which individuals should or should not undergo surgery was not included in the study. Therefore, it is not clear what value DNA analysis of cyst fluid added to patient management. It is not known if all of the malignant lesions may have been correctly sent to surgery without the information gained from the DNA testing or if the information gained by DNA analysis encouraged the evaluators to correctly assign participants to surgery. When reviewing the results of the PANDA trial, it is also important to consider the individual weight of each molecular marker in affirming a mucinous or malignant etiology. Although a number of markers were used in the DNA testing, the authors acknowledged it cannot be assumed that all markers are equally significant in the process of carcinogenesis and a much larger study is needed to address this question. There may be a role for the analysis of cyst fluid DNA in the evaluation of individuals with large lesions (in particular those having negative cytology and low CEA level) in which the identification of a pseudocyst may be difficult or there is clinical suspicion for a mucinous lesion. Because small lesions are likely to undergo surveillance, there may not be enough added value to DNA analysis of cyst fluid, especially in lesions that are less than 3 cm in size.

Rockacy and colleagues (2013) evaluated the association between KRAS mutation in pancreatic cyst fluid and the long-term outcomes of individuals. The authors collected data from 113 individuals with pancreatic cysts who underwent EUS with FNA at a tertiary care center from June 2004 to June 2007. Follow-up data were obtained through October 2010 (mean, 47 months). Pancreatic cysts were categorized as nonbenign or benign on the pathology results of surgical samples and participants' outcomes. The authors compared subject characteristics, presenting symptoms, EUS imaging characteristics, and results from analysis of cyst fluid, including cytology results, levels of CEA, and DNA sequencing results. A total of 51 participants underwent pancreatic surgery (10 malignant, 18 mucinous and 16 benign cysts). Sixty-three subjects were followed long-term, and 13 participants died of pancreatic cancer. On the basis of multivariate regression analysis, the presence of cyst solid component, participant symptoms, cyst size greater than 3 cm, and detection of KRAS mutations at codons 12 and 13 in cyst fluid were independently associated with a nonbenign course. While the authors concluded that KRAS mutations detected in pancreatic cyst fluid are associated with mucinous cysts which may progress to malignancy, in the discussion section of the article, the authors acknowledge that the characteristics of a solid cyst, greater than 3 cm in size and the presence of symptom features should not be weighed equally. Although the data in the study

support the resection of cysts that have a solid component or are symptomatic, the authors acknowledge resection of asymptomatic cystic lesions without a solid component because they are larger than 3 cm in size or manifest KRAS mutation, would lead to resection of many lesions that may otherwise have an indolent course. The authors encourage the safe monitoring of pancreatic cysts that are smaller than 3 cm in size and negative for the KRAS mutation. Limitations of the study include the fact that only cysts that underwent EUS-FNA were included in the study. Also, the classification design used in the study divided cysts into nonbenign and benign course categories based on the follow-up and may not be accurate in that cysts in the nonbenign course category may contain serous and retention cysts that grew in size during the follow-up period. Likewise, the benign course category may contain BD-IPMNs that did not grow during the follow-up period but still retain malignant potential. Also, the nonbenign course category may include mucinous cysts that would have remained stable during the course of the study had they not been resected.

A guideline published by the American College of Gastroenterology (ACG) states that a detailed molecular analysis of aspirated pancreatic cyst fluid may be helpful in predicting malignancy and that an initial KRAS mutation followed by allelic loss is most predictive (~90%) of a malignant pancreatic cyst. The guideline assigned the evidence for this indication as a level 3 indicating it was based on a published well-designed single group, cohort, time series or matched case-controlled studies (Khalid, 2007). However, the ACG guideline did not include KRAS mutation analysis in the formal recommendations set forth in this guideline. Similarly, in a more recent guideline on the diagnosis and management of pancreatic cysts, the ACG did not indicate that KRAS mutation testing should be used in the diagnosis or management of pancreatic cysts (Elta, 2018).

Gastric Cancer
Peer-reviewed published literature regarding the relationship between RAS status and gastric cancer is also limited. Hunt and colleagues (2001) explored if KRAS mutations were predictive for the development of gastric carcinoma. After 3 years, the data suggested that individuals whose baseline stomach biopsies revealed KRAS mutations were 3.74 times as likely to progress to a higher premalignant stage than those who lacked baseline mutations. However, at 6 years, baseline KRAS mutations failed to predict histological progression.

Endometrial Cancer
The available peer-reviewed studies evaluating the value of RAS status and endometrial cancer are also limited. Several small studies have suggested a relationship between KRAS mutations and the development of endometrial cancer (Caduff, 1995; Duggan, 1994; Fujimoto, 1993), but stopped short of identifying the exact role of KRAS mutations in the clinical setting. Estellar and colleagues (1997) analyzed KRAS point mutation and gene amplification in 55 endometrial carcinomas using polymerase to examine the prevalence and clinicopathological significance of KRAS oncogene activation in endometrial carcinoma and atypical hyperplasia. Point mutations at codon 12 of KRAS oncogene were identified in 8 of the 55 (14.5%) tumor specimens. The researchers did not detect any KRAS gene amplification in any of the endometrial carcinomas studied. No correlation was established between KRAS gene mutation and age at onset, histological subtype, grade of differentiation or clinical stage. The researchers concluded that KRAS mutation is a relatively common event in endometrial carcinomas, but did not provide any clear prognostic value.

Ovarian Cancer
The peer-reviewed literature regarding the role of RAS status in individuals with ovarian cancer has been predominately limited to small, non-randomized, uncontrolled studies specifically on the role of KRAS status. While these studies have demonstrated that there may be an association between KRAS mutations and the development of ovarian cancer, more research is needed to determine its exact role in the development of ovarian tumors (Cuatrecasas, 1997; Hogdall, 2003; Semczuk, 2004; Varraas, 1999).

Definitions

Adenocarcinoma: A type of cancer originating in cells that line specific internal organs and that have gland-like (secretory) properties.

Allele: One of a number of alternative forms of the same gene or same genetic locus (generally a group of genes).

Allelic loss: The loss of one of the two parental copies of an allele: also known as loss of heterozygosity.

Anal cancer: A type of cancer originating in the tissues of the anus; the anus is the opening of the rectum (last part of the large intestine) to the outside of the body.

Colon cancer: A type of cancer originating in the tissues of the colon (the longest part of the large intestine); most colon cancers are adenocarcinomas that begin in cells that make and release mucus and other fluids.

Colorectal cancer: A type of cancer originating in the colon (the longest part of the large intestine) or the rectum (the last several inches of the large intestine before the anus).

Epidermal Growth Factor Receptor (EGFR): A cell receptor that is associated with regulation of cell growth.

Gene amplification: A genetic variation characterized by the presence of multiple copies of the same genetic code on a chromosome.

KRAS status: Mutated: An altered DNA sequence within the KRAS gene, codons 12 or 13. Tumors with this KRAS type are much **less** likely to benefit from anti-EGFR therapy with cetuximab or with panitumumab.

KRAS status: Wild-type: The normal or typical form of the KRAS gene, as distinguished from any mutant forms. Tumors with this KRAS type are much **more** likely to benefit from anti-EGFR therapy with cetuximab or with panitumumab.

Metastatic: The spread of cancer from one part of the body to another; a metastatic tumor contains cells that are like those in the original (primary) tumor and have spread; also referred to as stage IV cancer.

Monoclonal antibody: A protein developed in the laboratory that can locate and bind to specific substances in the body, including on the surface of cancer cells.

Rectal cancer: A type of cancer originating in tissues of the rectum (the last several inches of the large intestine closest to the anus).

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Peer Reviewed Publications:

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Index

The use of specific product names is illustrative only. It is not intended to be a recommendation of one product over another, and is not intended to represent a complete listing of all products available.

History

Status	Date	Action
Revised	08/22/2019	Medical Policy & Technology Assessment Committee (MPTAC) review. Updated Title. In Clinical Indications section, revised Medically Necessary criteria to include NRAS, and revised Not Medically Necessary criteria to include all other indications for NRAS. Updated Description, Discussion/General Information, References, Websites for Additional Information, and Index sections. Updated Coding section; added 81311; also added 0111U effective 10/01/2019.
Reviewed	06/06/2019	MPTAC review. Updated Description, Discussion/General Information, References, and Websites for Additional Information sections.
Reviewed	03/21/2019	MPTAC review.
Reviewed	03/20/2019	Hematology/Oncology Subcommittee review. Updated the Discussion/General Information, References, Websites for Additional Information and History sections.
Reviewed	07/26/2018	MPTAC review.
Reviewed	07/18/2018	Hematology/Oncology Subcommittee review. Updated the Description, Discussion/General Information, References and History sections.
New	11/02/2017	MPTAC review.
New	11/01/2017	Hematology/Oncology Subcommittee review. Initial document development. Moved content of GENE.00014 to new clinical utilization management guideline document with the same title. Added the Praxis Extended RAS Panel to the Index section. Updated Coding section with 81479 (NOC) for extended RAS panel.