



The Pathologist's Message The importance of companion-test molecular assays for oncologic treatment decisions is on the rise. These tests are used to determine if patients are eligible to receive a targeted therapy. In the case of *KRAS* testing for colorectal cancer, the intent is to avoid unnecessary toxicity and monetary costs for patients who are not likely to respond to anti-EGFR therapies, by screening them prior to initiating therapy.

KRAS mutations can be detected in approximately 30-40% of all patients with CRC. Although no level I evidence* has been published, multiple studies with strong level II evidence have convincingly shown that patients with *KRAS* mutations in codons 12 or 13 do not benefit from anti-EGFR therapy with cetuximab or panitumumab. In contrast, about 40% of patients with metastatic colorectal cancer unresponsive to other therapies, and who lack a *KRAS* mutation, show a partial response with these agents. These findings suggest that only patients without *KRAS* mutations should be eligible to receive these therapies. See the ASCO Provisional Clinical Opinion www.asco.org/pco/kras.

Pathologist expertise is essential to quality KRAS testing and effective metastatic CRC patient treatment determination for the following reasons.

- *Identification of the right cells for assay analysis.* *KRAS* mutations are detected on DNA from tumor sections. A pathologist's evaluation of the tissue section used for DNA extraction is required to ensure that tumor cells are present in the specimen and that tumor cells are present in adequate quantity/concentration for the *KRAS* test that is utilized by the lab.
- *Consultation with oncologists and other members of the treatment team.* The pathologist can assist oncologists in the appropriate use of this test and guide the interpretation of results. Even if local pathologists are not performing the test in their own laboratory, they should understand the clinical significance of *KRAS* test results.
- *Reference laboratory selection.* Pathologists who utilize reference laboratories for this testing should be able to carefully evaluate the *KRAS* testing technology utilized and quality processes employed to ensure confidence in the results.
- *Technology selection.* Directors of molecular diagnostic laboratories will need to determine the best method for *KRAS* mutation detection in their environment.

***Note:** Levels of evidence in the literature are based on the classification developed by the U.S. Preventive Services Task Force. 1996. *Guide to Preventive Services*. 2d ed. Baltimore, MD: Williams and Wilkins. According to this classification, the quality of evidence can be scored as follows: (<http://www.ncbi.nlm.nih.gov/books/bv.fcgi?rid=hstat3.section.17745>)

- I. Evidence obtained from at least one properly designed randomized controlled trial.
- II-1. Evidence obtained from well designed controlled trials without randomization.
- II-2. Evidence obtained from well-designed cohort or case-controlled analytic studies, preferably from more than one center or research group.
- II-3. Evidence obtained from multiple time series with or without the intervention.
- III. Opinions of respected authorities, based on clinical experience, descriptive studies, or reports of expert committees.

Disclaimer: This POET was developed by the Technology Assessment Committee (TAC) with input from the Council on Scientific Affairs. Opinions expressed herein are solely those of the authors and do not represent those of the College of American Pathologists (CAP). No endorsement of any proprietary technology or product referenced is implied by the TAC or CAP. This report is provided for educational purposes only.

Clinical Context In the United States, an estimated 108,070 cases of colon and 40,740 cases of rectal cancer are expected to occur in 2008. Colorectal cancer is the third most common cancer and the second most common cause of cancer related deaths. An estimated 49,960 deaths from colon and rectum cancer are expected to occur in 2008, accounting for almost 9% of all cancer deaths.

Epidermal growth factor receptor (EGFR) has been validated as a therapeutic target in several human tumors. Recently, there has been a fair amount of interest in the role of anti-EGFR therapies in the treatment of CRC. EGF, as well as several other ligands, activates the cascade of RAS/RAF/MAPK, STAT, and PI3K/AKT signaling pathways when occupying the EGFR. The downstream effects of the activation influence cellular proliferation, adhesion, angiogenesis, migration, and survival.

This EGFR signaling pathway has received much attention for new drug development during the past 5-10 years because it is overexpressed in more than 85% of tumors from patients with metastatic colorectal cancer (mCRC).

Two relatively new anti-EGFR targeted antibodies, cetuximab and panitumumab, have shown promising activity as second-line therapy for mCRC and are used as first line therapy in combination with Oxaliplatin and Irinotecan. Cetuximab (Erbix[®]) was developed by Imclone and introduced in 2004. Panitumumab (Vectibix[®]) was developed by Amgen and introduced in 2006.

The anti-EGFR market is estimated at \$1.5 billion annually, with cetuximab at about \$1.2 billion and panitumumab at \$300 million.

Published clinical studies and studies presented at the 2008 American Society of Clinical Oncology (ASCO) annual meeting revealed that only patients whose tumors carried a wild-type (“normal”) sequence of the *KRAS* gene had a favorable response to cetuximab or panitumumab.

Technology Overview *KRAS* testing refers to the DNA-based assays that are used to detect mutations in the *KRAS* gene. *KRAS*, the human homolog of the Kirsten rat sarcoma-2 virus oncogene, encodes one of the proteins in the EGFR signaling pathway critical in the development and progression of cancer. *It is also known as Kras, K-RAS or KRAS2.*

- *KRAS* can harbor oncogenic mutations that yield a constitutively active protein in approximately 40% of CRC tumors.
- Most *KRAS* mutations occur in codon 12 or 13 of the gene.
- Recent studies have indicated that the presence of *KRAS* mutations in codon 12 or 13 is associated with a lack of response to EGFR inhibitors.
- The presence of *KRAS* mutations in the tumor is generally associated with a worse prognosis.
- *KRAS* mutations are also found in many other common tumor types beyond CRC, but their impact on anti-EGFR therapies have not yet been studied thoroughly.

Thus, patients with mutant *KRAS* (“abnormal”) will *not* benefit and are unnecessarily exposed to potential adverse events. The most common adverse reactions (incidence $\geq 25\%$) are: cutaneous (including rash, pruritus, and nail changes), headache, diarrhea, and infection. More serious adverse events such as severe allergic reaction and heart attack have been reported.

Awareness of *KRAS* testing for mCRC therapy guidance has spread rapidly among oncologists and gastroenterologists. At the same time, manufacturers of anti-EGFR therapies have been promoting the importance of *KRAS* mutation testing.

For practicing surgical pathologists, the potential magnitude of KRAS testing is significant. Several organizations have reviewed the evidence and have issued recommendations for *KRAS* testing in colorectal cancer patients. ASCO has recommended that all patients with metastatic colorectal cancer in whom EGFR antagonists are being considered should be tested for *KRAS* mutational status, at least involving codons 12 and 13. The National Comprehensive Cancer Network (NCCN) updated its Colon Cancer guideline with the recommendation that *KRAS* testing on the primary tumor or a site of metastasis should be part of the pre-treatment work-up for all mCRC patients (www.nccn.org). In July 2009, the Food and Drug Administration (FDA) approved labeling changes to cetuximab and panitumumab indicating that these agents are not recommended for the treatment of colorectal cancer harboring *KRAS* mutations.

It is also probable that this testing will be expanded to all tumors in which anti-EGFR therapy is being considered. Furthermore, it may be extended to other anti-receptor therapies which signal through *KRAS*. The impact on pathology practice workflow/costs will be minimal for laboratories already equipped with molecular diagnostic services. For laboratories performing the test, reimbursement should be on par with similar molecular tests.

Test Methods Used: Tests that are being used to assess for *KRAS* mutations are based on well-established molecular assays. These assays have generally been found to be sensitive, specific, and reliable.

A sample of the tumor is prepared and typically sent to a molecular diagnostic laboratory. The sample can be fresh, frozen or paraffin-embedded tissue depending on the methodology used. A pathologist needs to confirm that the submitted tissue specimen contains cancer cells and estimate the content of tumor cells (percentage tumor nuclei out of all nuclei present) in the specimen. This estimation of tumor content is important since different *KRAS* assays have different analytical sensitivities and an attempt should be made to enrich to a level that is acceptable for the assay being used. The DNA is then extracted.

Two commonly used methods to evaluate samples for *KRAS* mutations are:

- **Real-Time PCR.** In real-time PCR, fluorescent probes specific for the most common mutations in codons 12 and 13 are utilized. When a mutation is present, the probe binds and fluorescence is detected.
- **Direct Sequencing Analysis.** *KRAS* mutations can also be identified using a direct sequencing method of exon 2 in the *KRAS* gene. This technique identifies all possible mutations in the exon.

Acceleration/Deceleration Triggers to Adoption: The data supporting *KRAS* testing has brought to light other potential markers along the EGFR pathway as well as other pathways that may be important in predicting colorectal cancer patients' response to certain therapies. Likewise, since *KRAS* mutations are common in cancers other than CRC (e.g. pancreatic and lung cancer), several groups are now evaluating the role of *KRAS* in other cancers to see if the same concept holds true. Although rare, other *KRAS* mutations such as codon 61 mutations could be found to have clinical significance in the future. Mutations in other genes that act downstream of *KRAS* could be discovered.

It is likely that the momentum for clinical adoption of *KRAS* testing will be temporarily exponential. Sparked by ASCO 2008 at which the value of this testing was discussed, the number of *KRAS* tests ordered on a daily basis is increasing rapidly. As of September 2008, more than a dozen laboratories are already offering this test. Numerous forces are driving the interest in *KRAS* testing including:

- Multiple independent studies of cetuximab of mCRC patients in which response rates of 0% and shorter overall survival were observed among patients with *KRAS* mutations.
- The ability of *KRAS* testing to significantly reduce overall health care costs since cetuximab and panitumumab are very expensive drugs (estimated annual cost of \$100,000 each) and should not be utilized in patients who are not likely to respond.
- Promotion of *KRAS* testing as a tool in directing therapy by the pharmaceutical companies producing cetuximab and panitumumab.
- Publication of the ASCO Provisional Clinical Opinion specifying that *KRAS* testing should be done in metastatic colorectal cancer where EGFR antagonists are being considered. (www.asco.org/pco/kras)
- FDA's class labeling changes to anti-EGFR monoclonal antibodies, cetuximab (Erbix) and panitumumab (Vectibix): <http://www.fda.gov/AboutFDA/CentersOffices/CDER/ucm172905.htm>

Direct sequence analysis has lower analytical sensitivity than some of the real time PCR assays. However, the clinical relevance of a small percentage of cells with mutant *KRAS* has not been established.

Vendors: At this time, there is no FDA approved test for *KRAS* testing. *KRAS* testing can be performed using laboratory developed tests provided that the laboratory is accredited by the CAP or another CMS-deemed agency and has conducted the appropriate validation testing required by CLIA'88 regulations.

Multiple reference laboratories are now offering *KRAS* mutation testing. In addition, several vendors offer reagents for laboratory developed tests. It is expected that some of them will seek FDA approval for their assays.

Impact on Current Pathology Practice: For molecular diagnostic laboratories, *KRAS* testing could become an additional source of revenue that can offset fixed costs of running a molecular laboratory. Practices may need to expand their expertise in molecular techniques in order to capture this business. For these laboratories, minimal incremental investment would be required. However, in the short term until molecular testing in general grows, it is expected that most practices will send samples for *KRAS* testing to a reference laboratory.

For More Information: Peer Reviewed Published Literature**Clinical Trials**

1. Lievre A, Bachet JB, Le Corre D, et al: KRAS Mutation Status Is Predictive of Response to Cetuximab Therapy in Colorectal Cancer. *Cancer Res* 66: 3992-3995, 2006. <http://cancerres.aacrjournals.org/cgi/content/abstract/66/8/3992>
2. Di Fiore F, et al. Clinical relevance of KRAS mutation detection in metastatic colorectal cancer treated by cetuximab plus chemotherapy. *Br J Cancer* 96:1166-1169, 2007. <http://www.nature.com/bjc/journal/v96/n8/abs/6603685a.html>
3. Lièvre A, Laurent-Puig P. In Reply. *JCO* May 20 2008: 2601-2602. <http://www.jcojournal.org/cgi/reprint/26/15/2601>
4. Lievre, A., Bachet, J.-B., Boige, V., Cayre, A., Le Corre, D., Buc, E., Ychou, M., Bouche, O., Landi, B., Louvet, C., Andre, T., Bibeau, F., Diebold, M.-D., Rougier, P., Ducreux, M., Tomasic, G., Emile, J.-F., Penault-Llorca, F., Laurent-Puig, P. (2008). KRAS Mutations As an Independent Prognostic Factor in Patients With Advanced Colorectal Cancer Treated With Cetuximab. *JCO* 26: 374-379. <http://www.jcojournal.org/cgi/content/abstract/26/3/374>
5. Amado RG, Wolf M, Peeters M, Van Cutsem E, Siena S, Freeman DJ, Juan T, Sikorski R, Suggs S, Radinsky R, Patterson S, and Chang D. Wild-Type KRAS Is Required for Panitumumab Efficacy in Patients With Metastatic Colorectal Cancer. *J Clin Oncol* 26:1626-1634, 2008. <http://www.jco.ascopubs.org/cgi/content/abstract/26/10/1626>
6. De Roock W, et al. KRAS wild-type state predicts survival and is associated to early radiological response in metastatic colorectal cancer treated with cetuximab. *Ann Oncol* 19:508-515, 2008. <http://pt.wkhealth.com/pt/re/anon/abstract.00002352-200803000-00017>
7. Khambata-Ford S, et al. Expression of Epiregulin and Amphiregulin and K-ras Mutation Status Predict Disease Control in Metastatic Colorectal Cancer Patients Treated with Cetuximab. *J Clin Oncol* 25:3230-3237, 2008. <http://jco.ascopubs.org/cgi/content/abstract/25/22/3230>
8. Freeman DJ, et al. Association of K-ras mutational status and clinical outcomes in patients with metastatic colorectal cancer receiving Panitumumab alone. *Clin Colorectal Cancer* 7:184-190, 2008. <http://www.ncbi.nlm.nih.gov/pubmed/18621636>

Reviews/Opinion

9. Garassino MC, Farina G, Rossi A, Martelli O, and Rorri V. Should KRAS Mutations Be Considered an Independent Prognostic Factor in Patients With Advanced Colorectal Cancer Treated With Cetuximab? *JCO* May 20 2008: 2600. <http://jco.ascopubs.org/cgi/reprint/26/15/2600>
10. Downward J: Targeting RAS signaling pathways in cancer therapy. *Nat Rev Cancer* 3:11-22. <http://www.nature.com/nrc/journal/v3/n1/pdf/nrc969.pdf>
11. Raponi M, et al. KRAS mutations predict response to EGFR inhibitors. *Curr Opin Pharmacol* 8:1-6, 2008. <http://www.sciencedirect.com/science>
12. Tigue CC et al. The value of innovation: the economics of targeted drugs for cancer. *Targ Oncol* 2:113-119, 2007.

Methods for Mutation Detection

13. Nikiforova MN, Lynch RA, Biddinger PW, Alexander EK, Dorn GW 2nd, Tallini G, Kroll TG, Nikiforov YE. RAS Point Mutations and PAX8-PPAR Gamma Rearrangement in Thyroid Tumors: Evidence for Distinct Molecular Pathways in Thyroid Follicular Carcinoma. *J Clin Endocrinol Metab*. 2003 May;88(5):2318-26. <http://jcem.endojournals.org/cgi/content/abstract/88/5/2318>
14. Krypuy M, Newnham GM, Thomas DM, Conron M and Dobrovic A. High resolution melting analysis for the rapid and sensitive detection of mutations in clinical samples: KRAS codon 12 and 13 mutations in non-small cell lung cancer. *BMC Cancer* 2006, 6:295 doi:10.1186/1471-2407-6-295. <http://www.biomedcentral.com/1471-2407/6/295/>
15. Ogino S, et al. Sensitive Sequencing Method for KRAS Mutation Detection by Pyrosequencing. *J Mol Diagn* 7:413-421, 2005. <http://jmd.amjpathol.org/cgi/content/abstract/7/3/413>

Further Info

16. European Medicines Agency. Questions and Answers on the Marketing Authorisation for Vectibix. London, 20 September 2007. Doc. Ref. EMEA/405113/2007. www.emea.europa.eu/pdfs/human/opinion/40511307en.pdf
17. <http://www.kras-info.com>
18. Laboratories offering KRAS mutation testing: <http://www.amptestdirectory.org/>