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Q&A

By Joel A. Lefferts[1] and Gregory J. Tsongalis[2]

KRAS Mutation Detection: A New Look at an Old Gene

The introduction of small-molecule drugs and humanized monoclonal antibodies as novel therapies that target specific receptors and proteins in specific signal-transduction pathways has renewed interest in genes that had previously been identified as being involved in the tumorigenic process. The first of these genes was the *ERBB2*[3] [v-erb-b2 erythroblastic leukemia viral oncogene homolog 2, neuro/glioblastoma derived oncogene homolog (avian)] oncogene (also known as *HER2*), a member of the epidermal growth factor receptor (EGFR) family, which is amplified in human breast cancer. Diagnostic interests in this gene were never peaked until this receptor became the target of Herceptin, the first monoclonal antibody therapy against a cellular receptor. Similarly, EGFR has become the target of both monoclonal antibody therapies that target the binding domain and small-molecule drugs that inhibit its tyrosine kinase activity. *KRAS* (v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog) is a known oncogene that typically functions in the EGFR pathway. Although mutations in this oncogene are known to be associated with various types of human cancer, only recently has it become an attractive biomarker for the clinical laboratory and healthcare provider as a mechanism to determine therapeutic options. *KRAS* mutation detection has become part of the standard workup of patients with colorectal cancer, and those tumors that harbor a *KRAS* mutation have been shown to be nonresponsive to anti-EGFR therapies. The implementation of *KRAS* mutation testing in a clinical laboratory environment is surveyed below among 4 pathologists and a medical oncologist from 4 leading medical institutions in the US.

Is KRAS mutation testing performed routinely at your institution? If so, for which clinical indications? If not, what are the barriers that are affecting implementation?

Arief A. Suriawinata[4]: *KRAS* testing for colorectal cancer is routinely performed on patients who are being considered for anti-EGFR therapy including cetuximab and panitumumab at our institution. Oncologists are responsible for ordering the *KRAS* mutation analysis when they are considering anti-EGFR therapy as second-line treatment in patients with advanced colorectal cancer. The pathologist will then identify the appropriate tissue block and forward this to the Molecular Pathology Laboratory for testing. In addition, *KRAS* testing is performed on all lung adenocarcinoma cases.

William K. Funkhouser[5]: We perform *KRAS* mutation screening on a subset of cases with colorectal cancer and non–small cell lung cancer, generally from patients who are being considered for therapy with anti-EGFR antibodies (colorectal) or anti-EGFR tyrosine kinase inhibitors (lung).

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[1] Joel A. Lefferts, Postdoctoral Fellow, Molecular Pathology and Translational Research Program, Department of Pathology, Dartmouth Medical School, Dartmouth-Hitchcock Medical Center, Lebanon, NH.
[2] Gregory J. Tsongalis, Director of Molecular Pathology and Translational Research Program, Department of Pathology, Dartmouth Medical School, Dartmouth-Hitchcock Medical Center and Norris Cotton Cancer Center, Lebanon, NH.

[3] Human genes: *ERBB2*, v-erb-b2 erythroblastic leukemia viral oncogene homolog 2, neuro/glioblastoma derived oncogene homolog (avian); *KRAS*, v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog; *BRAF*, v-raf murine sarcoma viral oncogene homolog B1.

[4] Arief Suriawinata, Section Chief of Anatomic Pathology, Associate Professor of Pathology, Department of Pathology, Dartmouth-Hitchcock Medical Center, Lebanon, NH.
[5] William K. Funkhouser, Professor, Department of Pathology and Laboratory Medicine, Director of Anatomic and Surgical Pathology, UNC Hospitals, University of North Carolina, Chapel Hill, NC.

Marc Ladanyi[6]: Yes, we perform *KRAS* mutation testing routinely in 2 clinical settings: lung adenocarcinoma and metastatic colorectal carcinoma. In the former, all cases are tested for EGFR mutations first and, if negative, go on to *KRAS* testing. EGFR and *KRAS* mutations are mutually exclusive in lung adenocarcinoma. We test all resected lung adenocarcinomas since such a large proportion will recur. We test only metastatic colorectal carcinoma since, overall, most colorectal carcinomas are surgically curable.

Jan A. Nowak[7]: Currently, specimens are being sent to a reference laboratory for *KRAS* testing. Requests for *KRAS* testing come from oncologists who are determining treatment options for patients with colorectal carcinoma. Our laboratory is in the process of validating an assay for *KRAS* testing for implementation soon.

J. Marc Pipas[8]: At our institution, the GI Oncology Group is regularly performing *KRAS* mutation testing on all patients with metastatic colorectal cancer in whom anti-EGFR therapy is being considered. This is in keeping with the American Society of Clinical Oncology Provisional Clinical Opinion published in 2009. Given the lack of response to EGFR inhibitors in patients with *KRAS* gene mutations, anti-EGFR therapy is not appropriate in this group.

What types of treatment decisions are based on KRAS status, and are retrospective analyses of patients' tumors warranted?

Arief A. Suriawinata: The decision to treat patients with anti-EGFR therapy highly depends upon the *KRAS* gene mutation status; i.e., anti-EGFR therapy is administered only in patients with wild-type *KRAS*. Retrospective studies on tumor characteristics may be helpful in predicting the status of *KRAS* mutation and understanding specific histologic features associated with *KRAS* mutation; for example, our recent study in colorectal cancer showed that the majority of colorectal cancers with persistent preexisting villous adenoma were *KRAS* mutation positive.

William K. Funkhouser: Trial data showed that no colorectal carcinoma patient with an activating *KRAS* mutation has responded to treatment with anti-EGFR antibodies. Carcinomas from patients unresponsive to first-line therapy can be screened retrospectively for the presence of the activating *KRAS* point mutations in codons 12, 13, and 61 by use of DNA template extracted from paraffin blocks.

Marc Ladanyi: *KRAS* mutations are a very strong negative predictor of response to EGFR-targeted therapies in both lung adenocarcinoma and colorectal carcinoma. If no current sample is available for testing, retrospective analysis of a patient's initial surgical sample is useful, as *KRAS* mutations are typically present at diagnosis and are maintained throughout the course of the disease. Of course, the possibility of second or multiple primary cancers must be kept in mind.

Jan A. Nowak: Multiple studies have shown that patients with metastatic colorectal tumors that harbor *KRAS* codon 12 and 13 mutations do not benefit from treatment with cetuximab or panitumumab. Patients whose tumors have wild-type codon 12 and 13 sequences show a partial response to those agents. For patients who had been started on such therapy, *KRAS* testing would be useful in determining the potential of continued use of those agents.

[6] Marc Ladanyi, Attending Pathologist and Chief of Molecular Diagnostics Service, Department of Pathology, Member, Human Oncology and Pathogenesis Program, Memorial Sloan-Kettering Cancer Center, New York, NY.
[7] Jan A. Nowak, Director of Molecular Diagnostics Laboratory, Department of Pathology and Laboratory Medicine, Evanston Hospital NorthShore University HealthSystem, Evanston, IL.
[8] J. Marc Pipas, Associate Professor, Department of Medicine, Director GI Oncology Program, Dartmouth Medical School and Norris Cotton Cancer Center, Lebanon, NH.

J. Marc Pipas: Wild-type *KRAS* is necessary but not sufficient for response to EGFR inhibitors in patients with metastatic colorectal cancer. In addition, it has been shown that mutated *KRAS* [and *BRAF* (v-raf murine sarcoma viral oncogene homolog B1)] is associated with poorer overall survival. Since approximately 40% of tumor specimens in patients with colorectal cancer will exhibit *KRAS* mutations, *KRAS* mutation analysis is critical when considering anti-EGFR therapy. Patients with tumors exhibiting *KRAS* mutations should be considered for other treatments. Unfortunately, this does limit therapeutic options, but clinical trials are now open that specifically address patients with *KRAS*-mutated tumors.

Detection of somatic mutations in potentially heterogeneous tumors can be problematic. How does this affect your interpretation of these test results?

Arief A. Suriawinata: While we make the best effort to select a section that is most representative of the tumor for *KRAS* testing, we realize that, although rare, tumor heterogeneity can be a problem in large colorectal cancers with a few different lines of differentiation. Alternatively, *KRAS* testing can be performed in a metastasis that presumably represents the most aggressive clone if tumor heterogeneity is suspected. Our laboratory uses 5% controls for each of the 7 mutations that consist of wild-type and mutant synthetic oligonucleotides mixed at a 95:5 ratio, respectively. My concern with laboratories reporting the detection of multiple *KRAS* mutations in the same tumor is that this may reflect some technical challenges in assay performance due to the close proximity of the bases that are typically mutated (codons 12 and 13) vs true tumor heterogeneity.

William K. Funkhouser: Routine admixture of wild-type and mutant *KRAS* DNA implies a need for a high sensitivity for detection of the point mutations in codons 12, 13, and 61. We use spiked 5% positive controls and use pyrosequencing for detection of the point mutations. Sanger sequencing would be less sensitive, and melt-curve analysis would require confirmatory sequencing.

Marc Ladanyi: In our experience, key early driver genetic alterations such as *KRAS* mutations (or EGFR mutations) rarely if ever show genuine intratumoral heterogeneity. It is likely that most situations with apparent intratumoral heterogeneity for *KRAS* mutations actually represent testing of areas of differing tumor content, some of which may be below the sensitivity level of the testing method (typically Sanger sequencing). Other instances of heterogeneity for *KRAS* mutations may represent separate primaries. We have seen several patients with multiple primary lung adenocarcinomas, each containing a different mutation (EGFR or *KRAS*).

Jan A. Nowak: I am not aware that anyone has systematically addressed the issue of potential tumor heterogeneity with regard to *KRAS* mutation in colorectal tumors. Until there are good data, this will remain a hypothetical problem. We do know, however, that some 30%–40% of colon tumors will have *KRAS* mutations and that these tumors are not likely to respond to the anti-EGFR agents noted above. Assays that are similar in design and methodology should be as predictive as those used to establish the *KRAS*–EGFR observation. These are the assays laboratories are currently performing. Should tumor microheterogeneity be shown to be a factor, then the interpretation of *KRAS* test results will have to take that into account. Whether tumors that show significant *KRAS* heterogeneity will respond differently to anti-EGFR therapies is also unknown.

J. Marc Pipas: At our institution, we have experience with in-house testing for somatic *KRAS* mutations through use of real-time PCR and TaqMan probe chemistries. We have shown that this is a reliable, user-friendly, and robust method that can be used for low-volume testing using paraffin-embedded tissue specimens as well as material from fine-needle aspiration samples preserved in alcohol-based fixatives. We believe that this approach is reasonable for widespread clinical testing.

Do you think KRAS mutation testing will be extended to other tumor types?

Arief A. Suriawinata: Testing of other tumor types would only occur if the therapeutics can target this signal-transduction pathway in these other tumor types. Because EGFR also plays a significant role in other tumors, this scenario is highly likely.

William K. Funkhouser: *KRAS* mutation screening should be considered for any malignancy in which treatment of upstream kinases with antibodies or tyrosine kinase inhibitors is being considered. *BRAF* mutation screening should also be considered, since acti-

vating *BRAF* mutations could also preclude response to anti-EGFR therapy.

Marc Ladanyi: Currently, *KRAS* mutations are used as negative predictors of response to EGFR-targeted therapies, so the tumor types tested are those where EGFR-targeted therapies are already in widespread use, i.e., lung adenocarcinoma and colorectal carcinoma. However, targeted approaches to *KRAS* mutant cancers themselves are in various stages of clinical developmentand are likely to drive the expansion of *KRAS* testing across a wider range of tumor histologies.

Jan A. Nowak: It is likely. *KRAS* mutations are downstream activators of the EGFR signaling pathway, presumably the mechanism by which those mutations circumvent the efficacy of EGFR antagonists. In non–small cell lung cancer, *KRAS* mutations and EGFR mutations are reportedly mutually exclusive. It is likely that *KRAS* mutation testing will be part of the algorithm for evaluation of the EGFR pathway in non–small cell lung cancer.

J. Marc Pipas: *KRAS* testing, as well as testing of other components of the EGFR–MAPK signal-transduction pathway (such as *BRAF*), will continue to be evaluated in other tumor types and clinical scenarios. In addition, as other components of tumor metabolic pathways are discovered, these will certainly be pursued as possible prognostic and predictive factors in a variety of other cancers.

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