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Commentary

KRAS Mutations

An Old Oncogene Becomes a New Predictive Biomarker

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KRAS was one of the first genes found to be mutated in human cancer. For more than 20 years, mutations in the *KRAS* oncogene have been known to be present in a variety of human cancers, including lung cancer, colorectal cancer, and pancreatic cancer. The frequent mutation of this oncogene has driven efforts to develop a drug to target tumors with *KRAS* mutations. More recent data suggest that *KRAS* mutations are a useful biomarker of resistance to epidermal growth factor receptor (EGFR)-based therapeutics. Most commonly, predictive biomarkers are positive markers in which the presence of a change (increased protein, increased gene copy number, presence of a translocation, or a mutation) is correlated with the success of a particular therapy. A diagnostic marker that is now a predictive marker is perhaps the best example of this: the BCR-ABL translocation in chronic myelogenous leukemia. Patients with BCR-ABL are most likely to benefit from specific BCR-ABL inhibitors such as imatinib. Another clear forerunner exists in the area of breast cancer in which *ERBB2* amplification serves as a prognostic and predictive marker. However, for another class of agents, the EGFR inhibitors, *KRAS* mutations have recently emerged as a useful negative predictive biomarker, predicting when therapy with this class of targeted agents is unlikely to work.

Inhibitors of EGFR have been found to be effective in the treatment of several human cancers. The clinically useful EGFR inhibitors include kinase inhibitors such as erlotinib and gefitinib, as well as the anti-EGFR antibodies panitumumab and cetuximab. The kinase inhibitors have been most widely used in patients with lung adenocarcinoma. Erlotinib was approved by the U.S. Food and Drug Administration for use in the second and third line treatment of non-small cell lung cancer based on the results of a randomized placebo-controlled trial of patients with metastatic non-small cell lung cancer (unselected for *EGFR*- or *KRAS*-mutation status) that showed a response rate of 9% and an improvement in overall survival of about 2 months for patients treated with erlotinib.[1] Cetuximab and panitumumab, the anti-EGFR antibodies, are more widely used in colorectal cancer and cancers of the head and neck. Cetuximab is used alone and in combination with irinotecan for patients with advanced colorectal cancer based on demonstrated improvements in overall survival.[2,3] Panitumumab is generally used as a single agent in patients with colorectal cancer based on improvement in progression-free survival when compared with placebo.[4]

The positive predictive biomarkers for EGFR inhibitor therapy that have been explored include EGFR protein expression assayed by immunohistochemistry, *EGFR* copy number measured by chromogenic or fluorescence *in situ* hybridization, and *EGFR* mutations (reviewed in[5–7]). EGFR protein expression is the least specific marker for detecting patients likely to respond to therapy, with the majority of patients with lung adenocarcinoma expressing EGFR to some degree while only ~10% of patients respond to erlotinib or gefitinib. Moreover, EGFR immunohistochemical expression show little or no relationship to *EGFR* mutation status.[8] *EGFR* mutations are currently the most specific predictor of erlotinib or gefitinib response in patients with non-small cell lung cancer, with approximately 80% of patients with an activating mutation in *EGFR* going on to have a response to erlotinib or gefitinib. However, in retrospective series, there have been patients who responded to these therapies with negative results for all EGFR-related predictive biomarkers.[9,10] While it is possible that such discordant cases may reflect problems in the coverage or technical sensitivity of the *EGFR* mutation detection methods used, it is also clear that there is a need for other markers that might refine or complement response prediction in this context.

A complementary approach in the development of biomarkers is to identify factors that predict an absence of response, allowing physicians to prioritize therapies, re-

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Table 1. Retrospective Analyses of EGFR Tyrosine Kinase Inhibitors in Lung Adenocarcinoma

Author	Drugs	Patients tested for *KRAS* mutations (mutant/WT)	Response rate *KRAS* mutant	Response rate *KRAS* WT
Jackman[12]	Erlotinib	41 (6/35)	0%	14%
Zhu[13]	Erlotinib	206 (30/176)	5%	10%
Miller[9]	Erlotinib	80 (18/62)	0%	30%
Massarelli[14]	Erlotinib/Gefitinib	70 (16/54)	0%	7%
Hirsch[10]	Gefitinib	138 (36/102)	1%	7%
Hirsch[15]	Gefitinib	152 (12/140)	0%	8%
Han[16]	Gefitinib	69 (9/60)	0%	27%

WT: wild type (non-mutated).

ducing the chance that a patient will receive a therapy that is ineffective against their particular tumor. It is in this vein that *KRAS* mutation testing has come to the fore. In the bird's eye view of the EGFR pathway, signaling leads from the cell-surface receptor, via a number of signaling molecules, to growth and proliferation of cancer cells. One of the many signaling molecules downstream of EGFR is KRAS. It is because of this downstream role of KRAS that initial studies examining it as biomarker for resistance to EGFR-directed therapy hypothesized that mutations in *KRAS* would lead to cancer growth regardless of modulation of the EGFR signal (via kinase inhibition or antibodies to the receptor).[11]

Early work demonstrating that *KRAS* mutations can be a predictive biomarker for resistance to treatment with an EGFR inhibitor looked at a small number of patients with non-small cell lung cancer who had been treated with erlotinib or gefitinib, small molecule EGFR tyrosine kinase inhibitors. In this work, Pao and colleagues demonstrated that of those patients responsive to erlotinib or gefitinib, none (0/21) had *KRAS* mutations.[11] In contrast, 9/38 of patients refractory to erlotinib or gefitinib had *KRAS* mutations ($P = 0.02$). A number of groups have gone on to explore this relationship and confirmed that radiographical response to treatment with erlotinib or gefitinib is restricted to the population of patients with *KRAS* wild-type tumors (Table 1). These data were so compelling that many oncologists now routinely request *KRAS* mutation testing to identify patients who should be offered other therapies instead of erlotinib or gefitinib (Table 1).

The data supporting the use of *KRAS* mutation as a negative predictor of response are even more powerful in colorectal cancer. In colorectal cancer, a larger number of patient specimens have been examined for the negative predictive value of *KRAS* mutations for treatment with either cetuximab or panitumumab. In multiple single arm studies, investigators have demonstrated that the response rate for treatment with these EGFR antibodies is significantly greater in patients with tumors with wild-type *KRAS* as compared with mutated *KRAS.*[17–21] In trials of single-agent panitumumab or cetuximab, trials have demonstrated that the response rate of patients with *KRAS* mutations is 0%. Furthermore, in randomized controlled trials (Table 2), it is clear that *KRAS* mutations are not acting simply as a negative prognostic factor, but instead are negative predictors of response only to the EGFR inhibitors and not to conventional chemotherapy used in the treatment of colorectal cancer.

As a result of these relatively powerful clinical-molecular correlations, there is now a sudden increased demand for sensitive and rapid methods to detect the most common *KRAS* point mutations in routine clinical specimens. In this context, in this issue of *The Journal of Molecular Diagnostics*, Tatsumi et al report a novel assay for detection of mutations in codon 12 of *KRAS*.[25] Previously used methods to routinely detect *KRAS* mutations include direct sequencing, PCR-restriction fragment length polymorphisms, PCR-single strand conformation polymorphism, and mutant-allele-specific amplification. An even wider spectrum of technical approaches have already been applied to *EGFR* mutation detection.[26] Tatsumi et al have adapted the previously reported technology, smart amplification process and included a peptide nucleic acid clamp, developing an assay that can be rapidly performed without a separate DNA extraction. They report a very sensitive assay that can detect as few as 10 copies in 26 μL. More importantly, mutant DNA can be identified even when it comprises just 0.1% of the DNA in a specimen. This sensitivity could reduce or eliminate the need for microdissection, which is now commonly used to address the poor technical sensitivity of direct sequencing (about 25%). Moreover, a single assay in a single tube can be used to identify all possible mutations in codon 12 of *KRAS*, making it a very simple assay.

Table 2. Randomized Trials of EGFR Antibodies in Colorectal Cancer

Author	Drugs	Patients tested for *KRAS* mutations (mutant/WT)	Response rate *KRAS* mutant	Response rate *KRAS* WT
Amado[22]	Panitumumab	208 (84/124)	0%	17%
	Supportive care only	214 (100/114)	0%	0%
Van Cutsem[23]	FOLFIRI + cetuximab	277 (105/172)	36%	59%
	FOLFIRI	263 (87/176)	40%	43%
Bokemeyer[24]	FOLFOX + cetuximab	113 (52/61)	33%	61%
	FOLFOX	120 (47/73)	49%	37%

WT: wild type (non-mutated).

Recent clinical data have made clear that *KRAS* mutations are an important negative predictive biomarker. The technique reported by Tatsumi et al is an excellent example of technologies that are emerging that will allow rapid screening for *KRAS* mutations in clinical samples. However, as additional clinically relevant mutations in the EGFR pathway (for example in *BRAF* and *MEK1*[27,28]) and other targetable pathways continue to be discovered, the sheer number of mutations may lead to a move away from single mutation clinical assays to platforms which allow high level multiplexing.

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