Predicting Response to EGFR Inhibitors in Metastatic Colorectal Cancer: Current Practice and Future Directions

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Disclosures: Veena Shankaran: None; Jennifer Obel: None; Al B. Benson III: None.

The content of this article has been reviewed by independent peer reviewers to ensure that it is balanced, objective, and free from commercial bias. No financial relationships relevant to the content of this article have been disclosed by the authors or independent peer reviewers.

ABSTRACT

The identification of *KRAS* mutational status as a predictive marker of response to antibodies against the epidermal growth factor receptor (EGFR) has been one of the most significant and practice-changing recent advances in colorectal cancer research. Recently, data suggesting a potential role for other markers (including *BRAF* mutations, loss of phosphatase and tensin homologue deleted on chromosome ten expression, and phosphatidylinositol-3-kinase-AKT pathway mutations) in predicting response to anti-EGFR therapy have

emerged. Ongoing clinical trials and correlative analyses are essential to definitively identify predictive markers and develop therapeutic strategies for patients who may not derive benefit from anti-EGFR therapy. This article reviews recent clinical trials supporting the predictive role of *KRAS*, recent changes to clinical guidelines and pharmaceutical labeling, investigational predictive molecular markers, and newer clinical trials targeting patients with mutated *KRAS*. *The Oncologist* 2010;15:157–167

INTRODUCTION

Colorectal cancer (CRC) is the second leading cause of cancer death in the U.S. [1]. Despite improvements in CRC screening rates, an unfortunately large percentage of patients present with locally advanced or metastatic disease at the time of diagnosis [1]. Over the last decade, the development of novel therapeutics, including biologic agents such as antibodies that target the epidermal growth factor receptor (EGFR), has improved the prognosis for metastatic CRC (mCRC) patients. Recently, advances in our understanding of the EGFR pathway have led to the routine use of

Kirsten-ras (*KRAS*) mutational status as a predictive marker of response to anti-EGFR therapy.

Although EGFR is overexpressed in the majority (50%–80%) of colorectal tumors, it is clear that EGFR-targeted therapies are effective in only a subset of these patients. To use targeted drugs most effectively, it is important to identify rational molecular markers of response or lack of response to the therapy; such an approach optimizes the use of financial resources and prevents patients from receiving ineffective drugs. A well-known example of drug development paralleling discovery of rational molecular targets is

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trastuzumab, an anti-human epidermal growth factor receptor (HER)-2 monoclonal antibody. HER-2 is overexpressed in 10%–30% of malignant breast cancers, and overexpression of HER-2 is a strong predictor of response to trastuzumab therapy [2]. Since its approval by the U.S. Food and Drug Administration (FDA), trastuzumab use has been limited to patients whose tumors overexpress HER-2.

Like HER-2 overexpression in breast cancer, *KRAS* mutational status has emerged as a predictive molecular marker in CRC. Rigorous data have now clearly shown that activating *KRAS* mutations predict lack of response to anti-EGFR therapy. In fact, *KRAS* mutational status has also been shown to play a prognostic and predictive role in other tumor types, including lung cancer. This review highlights the major studies that have shown this correlation as well as the resulting changes to clinical guidelines and the FDA labeling for cetuximab and panitumumab. Further, the potential role of mutations at other points in the EGFR signaling pathway [including mutations in *BRAF*, loss of phosphatase and tensin homologue deleted on chromosome ten (PTEN) expression, and mutations in the phosphatidylinositol-3-kinase (PI3K)–AKT pathway] in predicting response to anti-EGFR therapy is discussed.

THE EGF MOLECULAR PATHWAY

EGFR, a 179-kDa transmembrane tyrosine kinase receptor, belongs to the HER family of cell surface receptors. Four structurally related proteins comprise the HER family: EGFR (HER-1/ErbB-1), HER-2 (ErbB-2), HER-3 (ErbB-3), and HER-4 (ErbB-4). Each receptor is composed of three domains: an extracellular domain that binds ligands, a lipophilic transmembrane segment, and an intracellular tyrosine kinase domain. Autocrine ligands such as EGF, amphiregulin (AREG), and epiregulin (EREG) trigger EGFR signaling by binding to its extracellular domain, causing dimerization and phosphorylation of the receptor. This process leads to activation of various intracellular signal transduction pathways, including the RAS–mitogen-activated protein kinase, PI3K–AKT, and signal transducer and activator of transcription (STAT) pathways (Fig. 1) [3–5]. Monoclonal antibodies such as cetuximab and panitumumab bind to EGFR with a high specificity, blocking ligand-induced phosphorylation of the receptor.

RAS proteins are members of a large superfamily of GTP-binding proteins that play a complex role in signal transduction of growth factor receptor–induced signals. The *KRAS* gene encodes one of these small GTP-binding proteins that acts as a signal transducer by cycling from GDP-bound to GTP-bound states in response to stimulation of EGFR. In its active GTP-bound state, RAS binds to key target proteins, which leads to activation of downstream

pathways. *KRAS* mutations result in constitutively active downstream signaling, even in the presence of anti-EGFR monoclonal antibodies [3–5].

KRAS AND ANTI-EGFR THERAPY: CLINICAL TRIALS

Although many other predictive molecular markers in oncology have been validated prospectively in clinical trials or studies as part of the drug development process, the validation of *KRAS* as a predictive molecular marker is based largely on retrospective data and correlative analyses of randomized studies. Though largely retrospective, the data supporting the predictive utility of *KRAS* are extensive and rigorous. Preliminary results from two randomized studies, however, have recently demonstrated a correlation between *KRAS* status and response to anti-EGFR therapy in a prospective fashion [6, 7].

Single-Arm Studies

KRAS mutational status was evaluated in relationship to response, progression-free survival (PFS), and overall survival (OS) in five single-arm studies of EGFR inhibitors in mCRC [8–12]. In all those studies, patients received second- or third-line EGFR inhibitors with or without chemotherapy. These small, post hoc analyses demonstrated a consistent correlation between the presence of a *KRAS* mutation and the lack of benefit from EGFR inhibitors (Table 1).

Randomized Controlled Trials

Seven large, randomized studies of EGFR inhibitors in mCRC have also undergone post hoc analyses to correlate outcome with *KRAS* mutational status. Those randomized studies were conducted in patients with refractory disease as well as in populations receiving first-line therapy for mCRC (Table 1).

Chemotherapy-Refractory Patients

Cetuximab and panitumumab have been shown to lead to longer PFS and OS times for patients with mCRC who have failed previous therapies. However, recent data have shown that this benefit is limited to those patients with wild-type (WT) *KRAS* status. Amado et al. [13] evaluated the predictive role of *KRAS* through a correlative analysis of a large phase III randomized trial comparing panitumumab monotherapy with best supportive care (BSC) in patients with chemotherapy-refractory disease. The BSC control arm allowed the authors to evaluate the relative effect of panitumumab therapy by *KRAS* mutational status independent of any potential prognostic effect of *KRAS* mutations. Of the 463 patients enrolled in the original randomized trial, 427 had adequate tissue samples for *KRAS* testing [13, 14].

Figure 1. EGFR signaling pathway. Ligands bind to EGFR and stimulate dimerization of the receptor. RAS is, in turn, activated, stimulating both the PI3K–AKT and RAF–MEK–MAPK pathways, resulting in cell proliferation, survival, and invasion.

Abbreviations: EGFR, epidermal growth factor receptor; GRB2, growth factor receptor-bound protein 2; HB-EGF, heparin-binding EGF-like growth factor; MAPK, mitogen-activated protein kinase; MEK, MAPK/extracellular signal-related kinase; PI3K, phosphatidylinositol-3-kinase; SOS, son of sevenless; TGF- α , transforming growth factor α .

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KRAS mutations were identified in 184 (43%) patients, including 84 in the panitumumab group and 100 in the BSC group. A longer PFS interval with panitumumab exposure was seen in the WT *KRAS* group (hazard ratio [HR], 0.45; 95% confidence interval [CI], 0.34–0.59); this same treatment effect was not seen in the mutant *KRAS* group (HR, 0.99; 95% CI, 0.73–1.36) [13].

In another phase III study, 572 patients with mCRC refractory to other therapies were randomized to either cetuximab or BSC [15]. Cetuximab treatment was associated with a greater median OS time than with BSC alone (6.1 months versus 4.6 months; HR, 0.77; 95% CI, 0.64–0.92; $p = .005$). In a subsequent correlative study from Karapetis et al. [16], *KRAS* mutational status was assessed in 394 of 572 patients originally included in the trial. Similar to other studies, cetuximab treatment was shown to result in longer PFS and OS times only in the WT *KRAS* group (OS, 9.5 months versus 4.8 months; HR, 0.55; 95% CI, 0.41–0.74; $p < .001$) and not in the *KRAS* mutant group [16].

First-Line Setting

Cetuximab Combined with Irinotecan in First-Line Therapy for Metastatic Colorectal Cancer (CRYSTAL)

Trial. The phase III CRYSTAL trial randomized 1,217 treatment-naïve mCRC patients to 5-fluorouracil, irinotecan, and leucovorin (the FOLFIRI regimen) with or without cetuximab [17]. Patients treated with the cetuximab-containing therapy were found to have a higher response rate (46.9% versus 38.7%; $p = .005$) and greater median PFS interval (8.9 months versus 8.0 months; $p = .036$). In a subsequent analysis of 540 patient samples from that study, *KRAS* mutations were seen in 35.6% of patients [18]. Although *KRAS* status did not seem to be of prognostic significance in the study (FOLFIRI-treated patients had the same outcome regardless of *KRAS* status), *KRAS* status did seem to predict responsiveness to cetuximab. In WT *KRAS* patients, cetuximab exposure was associated with a better therapeutic response (59.3% versus 43.2%; $p = .0025$) and

Table 1. Correlative analyses of *KRAS* status with response to anti-EGFR antibodies in mCRC

Study	Study population	Outcome measure	WT <i>KRAS</i>	Mutant <i>KRAS</i>
Single-arm studies				
Lievre et al. [12]	89 patients treated with C, C + I, or C + 5-FU + I	<i>n</i>	65	24
		RR	40%	0%
		PFS	31.4 wks	10.1 wks
		OS	14.3 mos	10.1 mos
De Roock et al. [9]	113 patients treated with C or C + I	<i>n</i>	67	46
		RR	41%	0%
		PFS	43 wks	27.3 wks
		OS	74.9 wks	30.6 wks
Khambata-Ford et al. [11]	110 patients treated with C alone	<i>n</i>	50	30
		RR	10%	0%
Di Fiore et al. [10]	59 patients treated with C + chemotherapy	<i>n</i>	43	16
		RR	10%	0%
Benvenuti et al. [8]	48 patients treated with C, P, or C + chemotherapy	<i>n</i>	32	16
		RR (<i>p</i> = NS)	31%	6%
Study	Study population	% <i>KRAS</i> mutants	WT <i>KRAS</i>	Mutant <i>KRAS</i>
Randomized studies				
Amado et al. [13]	463 patients randomized to BSC versus P	43%	Median PFS, 12.3 wks (P) versus 7.3 wks (BSC); <i>p</i> < 0.0001 Median OS: HR, 0.99 (95% CI, 0.75–1.29; <i>p</i> = NS)	Median PFS: 7.4 wks (P) versus 7.3 wks (BSC), <i>p</i> = NS Median OS: HR 1.02 (95% CI 0.75–1.39, <i>p</i> = NS)
Karapetis et al. [16]	572 patients randomized to BSC versus C	42.3%	Median PFS, 3.7 mos (C) versus 1.9 mos (BSC); <i>p</i> < .001 Median OS, 9.5 mos (C) versus 4.8 mos (BSC); <i>p</i> < .001	Median PFS, 1.8 mos (C) versus 1.8 mos (BSC); <i>p</i> = .96 Median OS, 4.5 mos (C) versus 4.6 mos (BSC); <i>p</i> = .98
Van Cutsem et al. [18] (CRYSTAL)	1,127 patients randomized to first-line FOLFIRI with or without C	35.6%	Median PFS: HR, 0.68 (95% CI, 0.051–0.934; <i>p</i> = .0167) favoring C	Median PFS: HR, 1.07 (95% CI, 0.71–1.61; <i>p</i> = .75)
Maughan et al. [19] (MRC COIN)	1,630 patients randomized to first-line FOLFOX or CapOx with or without C	43%	Median OS, 17.0 mos (FOLFOX/CapOx + C) versus 17.9 mos (FOLFOX/CapOx alone); HR, 1.038; <i>p</i> = .68	Not analyzed
Bokemeyer et al. [21] (OPUS)	337 patients randomized to first-line FOLFOX with or without C	42%	Median PFS, 7.7 mos (FOLFOX + C) versus 7.2 mos (FOLFOX); <i>p</i> = .02	Median PFS, 5.5 mos (FOLFOX + C) versus 8.6 mos (FOLFOX); <i>p</i> = .02
Tol et al. [23] (CAIRO-2)	755 patients randomized to CapOx and B with or without C	39.6%	Median PFS, 10.5 mos (CapOx + B + C) versus 10.6 mos (CapOx + B); <i>p</i> = .30 Median OS, 21.8 mos (CapOx + B + C) versus 22.4 mos (CapOx + B); <i>p</i> = .64	Median PFS, 8.1 mos (CapOx + B + C) versus 12.5 mos (CapOx + B); <i>p</i> = .003 Median OS, 17.2 mos (CapOx + B + C) versus 24.9 mos (CapOx + B); <i>p</i> = .03

(continued)

Table 1. (Continued)

Study	Study population	% <i>KRAS</i> mutants	WT <i>KRAS</i>	Mutant <i>KRAS</i>
Hecht et al. [24] (PACCE)	1,053 patients randomized to FOLFOX or FOLFIRI with B with or without P	40%	Median PFS, 9.8 mos (FOLFOX + B + P) versus 11.5 mos (FOLFOX + B); 10 mos (FOLFIRI + B + P) versus 12.5 mos (FOLFIRI + B)	Median PFS, 10.4 mos (FOLFOX + B + P) versus 11.0 mos (FOLFOX + B); 8.3 mos (FOLFIRI + B + P) versus 11.9 mos (FOLFIRI + B)

Abbreviations: 5-FU, 5-fluorouracil; B, bevacizumab; BSC, best supportive care; C, cetuximab; CAIRO-2, Capecitabine, oxaliplatin and bevacizumab with or without cetuximab in first-line advanced colorectal cancer; CapOx, capecitabine and oxaliplatin; CI, confidence interval; COIN, Continuous chemotherapy plus cetuximab or intermittent chemotherapy with standard continuous palliative combination chemotherapy with oxaliplatin and a fluoropyrimidine in first-line treatment of metastatic colon cancer; CRYSTAL, Cetuximab combined with irinotecan in first-line therapy for metastatic colorectal cancer; EGFR, epidermal growth factor receptor; FOLFIRI, 5-FU, leucovorin, and irinotecan; FOLFOX, 5-FU, leucovorin, and oxaliplatin; HR, hazard ratio; I, irinotecan; mCRC, metastatic colorectal cancer; MRC, Medical Research Council; NS, not significant; OPUS, Oxaliplatin and cetuximab in first-line treatment of mCRC; OS, overall survival; P, panitumumab; PACCE, Panitumumab advanced colorectal cancer evaluation study; PFS, progression-free survival.

a longer median PFS interval (HR, 0.68; 95% CI, 0.051–0.934; $p = .0167$). For *KRAS* mutants, there was no significant difference in terms of response or median PFS with cetuximab exposure; in fact, there was a trend toward a shorter PFS time in the cetuximab arm (HR, 1.07; 95% CI, 0.71–1.61; $p = .46$) [18].

Medical Research Council COIN Trial. Conversely, preliminary results from the Medical Research Council phase III Continuous Chemotherapy plus Cetuximab or Intermittent Chemotherapy with Standard Continuous Palliative Combination Chemotherapy with Oxaliplatin and a Fluoropyrimidine in First-Line Treatment of Metastatic Colon Cancer (COIN) study failed to demonstrate a benefit with first-line cetuximab therapy, even in WT *KRAS* patients [19]. In that study, 1,630 patients with mCRC were randomized to a fluoropyrimidine (either 5-fluorouracil plus folinic acid or capecitabine, per patient and physician choice) plus oxaliplatin alone or in combination with cetuximab. Though both mutant and WT *KRAS* patients were accrued to the study, the primary analysis conducted in the WT *KRAS* group revealed no difference in terms of the median OS time with the addition of cetuximab (17.0 months versus 17.9 months; HR, 1.038; $p = .68$) [19].

OxaliPlatin and cetUximab in firSt-line Treatment of mCRC (OPUS) Trial. The randomized phase II OPUS trial evaluated the benefit of 5-fluorouracil, oxaliplatin, and leucovorin (the FOLFOX regime) with or without cetuximab in the first-line treatment of mCRC patients [20, 21]. In total, 337 patients were randomized in that study; neither the PFS time nor the overall response rate was significantly

different with the addition of cetuximab. In a secondary analysis correlating efficacy with *KRAS* status, 233 of the 337 patient specimens were eligible for *KRAS* testing; *KRAS* mutations were found in 42% of cases. Patients with WT *KRAS* were found to have a higher response rate (61% versus 37%; $p = .011$) and lower risk for progression (HR, 0.57; $p = .016$) when exposed to cetuximab. *KRAS* mutants, conversely, had a higher risk for disease progression with exposure to FOLFOX plus cetuximab, compared with FOLFOX alone (HR, 1.83; 95% CI, 1.095–3.056; $p = .0192$). These findings suggest a potential negative effect when cetuximab is administered to patients whose tumors carry *KRAS* mutations.

Dual Biologic Therapy, First-Line Setting

CAIRO Study. The Capecitabine, Oxaliplatin and Bevacizumab with or without Cetuximab in First-Line Advanced Colorectal Cancer (CAIRO-2) trial was a randomized phase III study designed to investigate the role of dual biologic therapy in the first-line treatment of mCRC patients [22, 23]. All patients received capecitabine and oxaliplatin combined with bevacizumab, an antibody against vascular endothelial growth factor (VEGF); patients were randomized to this regimen with or without cetuximab. At the time of an interim analysis, patients who did not receive cetuximab had a longer PFS duration than patients who did receive cetuximab (10.7 months versus 9.8 months; HR, 1.22; 95% CI, 1.03–1.44; $p = .019$) [22, 23]. There was no difference in terms of OS between the two groups. In a subgroup analysis, *KRAS* status was assessed in 528 tumor specimens; *KRAS* mutations were found in 39.6% of cases. Although

WT *KRAS* patients had comparable outcomes regardless of treatment arm, *KRAS* mutants experienced a significantly shorter PFS interval (8.1 months versus 12.5 months; $p = .003$) and OS time (17.2 months versus 24.9 months; $p = .03$) when exposed to the cetuximab-containing treatment arm. This analysis similarly showed a potential deleterious effect of cetuximab in patients with *KRAS* mutations.

Panitumumab Advanced Colorectal Cancer Evaluation

(PACCE) Study. Like the CAIRO-2 trial, the PACCE phase III randomized study similarly investigated the role of dual biologic therapy in the first-line treatment of mCRC patients. In this study, patients were treated with cytotoxic chemotherapy (a fluoropyrimidine plus oxaliplatin or a fluoropyrimidine plus irinotecan, depending on the physician's choice) and bevacizumab with or without the addition of panitumumab [24]. In the cohort receiving a fluoropyrimidine plus oxaliplatin plus bevacizumab with or without panitumumab, combined antibody therapy resulted in a higher risk for progression (PFS time, 8.8 months versus 10.5 months; HR, 1.44; 95% CI, 1.13–1.85; $p = .004$). In the group receiving a fluoropyrimidine plus irinotecan plus bevacizumab with or without panitumumab, there was a trend toward a shorter PFS time in the combined antibody group, though this was not statistically significant. In both cohorts, toxicity and the number of adverse events were strikingly higher with the addition of panitumumab. In a secondary analysis, *KRAS* mutations were found in 40% of the 865 specimens tested. As seen in other studies, *KRAS* mutants did not do better when exposed to the panitumumab-containing arm (in fact, there was a trend toward shorter PFS and OS times with combined antibody therapy). Interestingly, however, patients with WT *KRAS* (in the fluoropyrimidine plus oxaliplatin-treated cohort) had shorter PFS and OS times with combined antibody therapy than with the control treatment (median OS time, 20.7 months versus 24.5 months; HR for progression, 1.89; 95% CI, 1.30–2.75), suggesting a potential negative interaction between anti-EGFR and anti-VEGF therapy [24].

Randomized Controlled Trials:

Prospective Studies

Two Amgen-sponsored trials (the Panitumumab Randomized Trial in Combination with Chemotherapy for Metastatic Colorectal Cancer to Determine Efficacy [PRIME] and 181 trials) attempted to demonstrate the prospective predictive utility of *KRAS* in first-line and second-line treatment of mCRC patients. Preliminary results from both studies were presented at the 2009 European Society for Medical Oncology meeting [6, 7]. In the multicenter, phase III PRIME study, 1,183 previously untreated patients with

mCRC were randomized to first-line FOLFOX4 with or without panitumumab; *KRAS* status was determined prospectively, and this gene was found to be mutated in 40% of patients [6]. Patients with mutated *KRAS* randomized to FOLFOX4 with panitumumab had a shorter median PFS interval than patients randomized to FOLFOX4 alone (7.3 months versus 8.8 months; HR, 1.29; 95% CI, 1.04–1.62; $p = .0227$). Conversely, patients with WT *KRAS* status had a longer median PFS time with panitumumab treatment (9.6 months versus 8.0 months; HR, 0.80; 95% CI, 0.66–0.97; $p = .0234$). The multicenter, phase III 181 study randomized 1,186 patients with mCRC to second-line therapy with FOLFIRI with or without panitumumab [7]. *KRAS* status was similarly determined in a prospective fashion. Although no benefit was associated with panitumumab use in patients with mutated *KRAS*, patients with WT *KRAS* had a longer median PFS time (5.9 months versus 3.9 months; HR, 0.73; 95% CI, 0.593–0.903; $p = .004$) with exposure to panitumumab; the difference in the median OS time was not statistically significant. These are the first studies to demonstrate a correlation between *KRAS* status and response to anti-EGFR therapy in a prospective fashion.

GUIDELINES

In January 2009, the American Society of Clinical Oncology (ASCO) issued a provisional clinical opinion (PCO) regarding the utility of testing for mutations in codons 12 or 13 of the *KRAS* gene in patients with mCRC to predict responsiveness to EGFR inhibitors [25, 26]. A PCO is designed to offer clinical direction to ASCO membership following publication or presentation of potentially practice-changing data derived from rigorous studies.

PCO: Based on a systematic review of the relevant literature, all patients with mCRC who are candidates for anti-EGFR antibody therapy should have their tumor block tested for *KRAS* mutations in a clinical laboratory improvement amendments (CLIA)-accredited laboratory. If a *KRAS* mutation in codon 12 or 13 is detected, patients should not receive anti-EGFR antibody therapy as part of their treatment [25, 26].

Updated National Comprehensive Cancer Center Network (NCCN) CRC guidelines also strongly recommend *KRAS* genotyping on tumor tissue in all patients with mCRC to guide decision making regarding the use of EGFR inhibitors. The guidelines clearly state that patients with known *KRAS* mutations in codons 12 and 13 should not receive cetuximab or panitumumab, either alone or in combination with other drugs, because the exposure to toxicity and expense cannot be justified [27].

European regulatory authorities, including the European Committee for Medicinal Products for Human Use, have approved EGFR inhibitors only for those mCRC pa-

tients with WT *KRAS* [28]. One of the U.S.'s largest private insurance companies, Blue Cross Blue Shield, also issued a statement endorsing the lack of clinical benefit of EGFR inhibitors in *KRAS* mutant tumors [29]. As of July 17, 2009, the FDA has changed the labeling for cetuximab and panitumumab to limit the use of these agents to patients with WT *KRAS* tumors [30].

METHODS FOR DETECTING *KRAS* MUTATIONS IN CRC

Despite recent labeling changes limiting the use of anti-EGFR monoclonal antibodies to patients with WT *KRAS* tumors, there are currently no FDA-approved *KRAS* mutational assays available on the market. Pathologists and CLIA-certified laboratories therefore play an instrumental role in quality assurance for *KRAS* testing, particularly given the rapidly increasing clinical demand for this assay.

Although the dual nature of mutation analysis (i.e., either “present” or “absent”) may decrease subjectivity in test interpretation, there are numerous other issues to consider pertaining to tissue acquisition, detection, and reporting of *KRAS* mutations [31, 32]. ASCO and the College of American Pathologists (CAP) have both endorsed basic guidelines to ensure optimal assessment of *KRAS* mutations [25, 26, 33].

Tissue Processing

Both fresh-frozen tissue and formalin-fixed paraffin-embedded tissue blocks are considered to be acceptable for analysis both by ASCO and CAP [25, 33]. Formalin fixation has been shown to result in DNA degradation; in order to ensure accurate testing, it has therefore been recommended that a total of 30 ng DNA be obtained [31]. Because excessive surrounding necrosis and adjacent reactive cells in the specimen can impede tumoral *KRAS* evaluation, careful examination and, in some cases, microdissection by the pathologist are essential to ensure optimal testing [31, 32].

Detection of Gene Mutation and Reporting of Results

Although mutations have been reported to occur in various codons (including codons 61 and 146) within the *KRAS* gene, the majority of mutations occur in codons 12 and 13 of exon 2 [31, 34]. Various techniques have been described to detect these mutations; however, the two recommended and most commonly used techniques include direct sequencing and allele-specific real-time polymerase chain reaction (PCR) techniques. In the former approach, the entire genomic region (e.g., exon 2) is amplified, fully sequenced, and compared with a normal sequence. The latter approach involves the use of primers specifically corresponding to

known mutations in codons 12 and 13. Both approaches have advantages and drawbacks. Whereas direct sequencing has a high specificity, it has a relatively low sensitivity, slower turnaround time, and may require a larger amount of DNA specimen. Allele-specific real-time PCR is a much quicker test with a higher sensitivity than direct sequencing, but may miss more unusual mutations in the *KRAS* gene [31]. Once the tests are performed, results should be reported reliably and consistently; the role of the pathologist in reporting the assay used and the limitations of these assay techniques cannot be overlooked. All the previously mentioned retrospective analyses of randomized anti-EGFR trials preferentially used the allele-specific real-time PCR technique to determine *KRAS* mutational status (Table 1) [13, 16, 18, 21, 23, 24].

Testing Primary Tumor Versus Metastatic Lesions

Current evidence suggests a high concordance between *KRAS* mutational status in the primary tumor and in the metastatic lesion. In a recent analysis of 107 primary CRC lesions and corresponding metastatic lesions, concordance in *KRAS* mutation assay results between primary and metastatic lesions was seen in 96% of cases [35]. Testing either the primary tumor or the metastatic lesion are both considered acceptable approaches.

Which Patients Should Undergo Testing?

While both the ASCO and NCCN endorse routine *KRAS* testing of any patient with mCRC being considered for anti-EGFR therapy, some physicians and centers have started to adopt routine *KRAS* testing for patients with newly diagnosed metastases or patients with stage III disease as a means of future treatment planning and prognostication [32]. As the role of *KRAS* mutations is further investigated, routine inclusion of this test in the pathologic review of earlier stage CRC may one day play a role. Still, the utility of routine *KRAS* testing in earlier stage disease is of uncertain significance given the lack of evidence to support the routine use of anti-EGFR therapy in the adjuvant setting.

INVESTIGATIONAL MOLECULAR MARKERS AND RESPONSE TO EGFR INHIBITORS

In addition to *KRAS*, the role of other potential markers in predicting response to anti-EGFR therapy is currently being investigated. These markers include *BRAF* mutations, PTEN expression, other PI3K–AKT pathway mutations, and expression of EGFR ligands. Particularly interesting is the utility of these markers in predicting resistance to EGFR inhibitors in WT *KRAS* tumors.

The Role of *BRAF* Mutations

In the RAS–RAF–mitogen-activated protein kinase/extracellular signal–related kinase kinase (MEK) pathway of the EGFR receptor, *BRAF* (B-type Raf kinase) is the primary downstream effector of *KRAS* signaling [36]. Though *BRAF* and *KRAS* mutations have been shown to be mutually exclusive events, *BRAF* may have prognostic and predictive significance in WT *KRAS* tumors [37, 38].

In a recent analysis by Loupakis et al. [34], 87 patients with WT *KRAS* tumors receiving cetuximab and irinotecan for refractory mCRC underwent evaluation for the *BRAF* V600E mutation and *KRAS* codon 61 and 146 mutations; the presence of these mutations was correlated with PFS and OS. *BRAF* V600E mutations were found in 15% of the 87 patients analyzed; patients with this mutation were found to have a lower response rate to therapy (0% versus 32%; $p = .016$) and shorter OS time (4.1 months versus 13.9 months; $p = .037$). In another recent retrospective analysis of 113 patients treated with cetuximab or panitumumab, *BRAF* mutations were found in 14% of the WT *KRAS* patients [37]. None of the patients with *BRAF* mutations responded to anti-EGFR therapy. Further, patients with *BRAF* mutations had shorter PFS ($p = 0.011$) and OS ($p < .001$) times than WT *BRAF* cases. In a concurrent analysis of CRC cell lines, the *BRAF* V600E mutation was similarly shown to impair the efficacy of cetuximab and panitumumab; interestingly, response to these agents was restored by the addition of sorafenib, an inhibitor of *BRAF*. *BRAF* status was also shown to have potential prognostic significance in a recent retrospective analysis of 516 patients treated with chemotherapy and bevacizumab with or without cetuximab [36]. In that analysis, *BRAF* mutations were associated with shorter PFS and OS times regardless of exposure to cetuximab.

Like mutations in *BRAF*, mutations in *NRAS* (a *ras* oncogene variant) may also have predictive utility in WT *KRAS* tumors. In a recent report from Lambrechts et al. [39], *NRAS*, *BRAF*, and *KRAS* mutations were all found to be mutually exclusive events, with combined WT *KRAS*–*BRAF*–*NRAS* tumors associated with a higher response rate and longer PFS time. Conversely, patients with WT *KRAS* tumors did not respond to cetuximab therapy when a mutation in *NRAS* was present. [39] On the other hand, some data suggest that WT *KRAS* patients with high *EGFR* gene copy number (GCN) expression may be particularly sensitive to anti-EGFR therapy [40, 41].

Although *BRAF* mutational status is not routinely being used to determine the use of EGFR inhibitors, emerging data seem to suggest a predictive role for *BRAF* mutations in WT *KRAS* tumors. Because only a fraction of WT *KRAS* tumors respond to anti-EGFR therapy, consideration of

other factors that may mediate resistance is important. Ongoing investigation into the potential predictive role of *NRAS* and *EGFR* GCN may also help with treatment planning in WT *KRAS* patients.

PTEN and the PI3K–AKT Pathway

Whereas the RAS–RAF–MEK pathway controls cell proliferation, the PI3K–AKT pathway controls cell survival and motility in response to EGFR signaling. PTEN regulates the PI3K–AKT pathway such that loss of PTEN expression contributes to further activation of this pathway. Several studies have investigated whether loss of PTEN expression and mutations in the PI3K–AKT pathway may, in turn, confer resistance to EGFR inhibitors [42–49].

In one of the earliest studies to describe a correlation between loss of PTEN and resistance to anti-EGFR therapy, 27 cetuximab-treated patients were analyzed for PTEN expression by immunohistochemistry [42]. Response to cetuximab was observed in 0% of patients with lack of PTEN expression and 37% of those with intact PTEN expression, suggesting that PTEN loss may mediate resistance to EGFR inhibitors. In a subsequent analysis of 22 CRC cell lines, loss of PTEN expression and *PIK3CA* mutations were associated with resistance to cetuximab; the combination of these abnormalities with either *KRAS* or *BRAF* mutations resulted in maximal resistance to cetuximab, compared with those lacking simultaneous mutations (38.8% versus 10.8% growth inhibition; $p = .002$) [43]. In a recent retrospective analysis from Laurent-Puig et al. [50], *BRAF* mutations and loss of PTEN expression were both associated with a shorter OS time ($p < .001$ and $p < .13$, respectively) in a group of WT *KRAS* patients treated with cetuximab-based therapy. Although a retrospective analysis from Prenen et al. [47] failed to confirm a correlation between *PIK3CA* mutations and resistance to cetuximab within a group of 200 chemorefractory mCRC patients, other studies have supported this association [48, 49]. The clinical utility of these assays, however, remains unclear, and they are not part of routine clinical practice.

The novel use of agents such as inhibitors of the mammalian target of rapamycin (mTOR) has been of recent interest. The mTOR complex is the main downstream effector of the PI3K–AKT pathway; drugs that block mTOR signaling may, in turn, overcome the activating effects of PTEN loss or PI3K–AKT pathway mutations [51]. Although mTOR inhibitors are FDA approved for use in renal cell cancer, their role in the treatment of CRC is investigational. Recent data suggest that mTOR inhibitors may be of benefit in combination with chemotherapeutics or EGFR inhibitors. Such an approach was shown to over-

come resistance to EGFR inhibition in vitro [52]. An ongoing phase II clinical trial from Denmark is currently investigating the role of mTOR inhibition with temsirolimus in patients with *KRAS* mutated, refractory CRC [53]. Further investigation of agents that may potentially overcome mutations in the PTEN–PI3K–AKT pathway could be of great promise.

The Role of EGFR Ligands

Although the degree of EGFR expression has not been shown to correlate with response to EGFR inhibitors, expression of the EGFR ligands EREG and AREG has been shown to correlate with response. In a study from Khambata-Ford et al. [11], tumor specimens from 110 patients receiving cetuximab monotherapy were analyzed by gene expression profiling; *KRAS* status and EREG/AREG expression emerged as potential indicators of therapeutic response. Patients with high *EREG* and *AREG* gene expression had a longer PFS time than patients with low expression (*EREG*, 103.5 days versus 57 days; $p = .0002$ and *AREG*, 115.5 days versus 57 days; $p < .0001$). Two reports recently demonstrated that the predictive power of EGFR ligand expression may be greatest among patients with WT *KRAS* [54, 55]. In a correlative analysis of specimens from 385 patients treated with either cetuximab or BSC, patients with tumors with the “combimarker” of high *EREG* expression and WT *KRAS* had the longest PFS and OS times when treated with cetuximab [54]. Finally, in a recent analysis of EGFR ligand expression in 220 WT *KRAS* patients with refractory mCRC receiving irinotecan and cetuximab, the OS time was longer in patients with high *EREG* (HR, 0.42; 95% CI, 0.28–0.63; $p < .0001$) and high *AREG* (HR, 0.40; 95% CI, 0.27–0.64; $p < .0001$) expression. The same trend was not observed in patients with *KRAS* mutated tumors [56].

Although no markers besides *KRAS* have yet been validated and endorsed as predictive markers for response to EGFR inhibitors, further investigation is necessary. These markers may help to optimize treatment planning in WT *KRAS* patients.

ONGOING CLINICAL TRIALS

Issues and Amendments to Previous Clinical Trials

Because of the large amount of data supporting the predictive role of *KRAS* as well as recent changes to ASCO and NCCN guidelines, several ongoing clinical trials have been modified to reflect these data. Many newer trials involving EGFR inhibitors have also incorporated WT *KRAS* status in the eligibility criteria. Two large, high-profile trials that

have been added accordingly include the Gastrointestinal Intergroup trial North Central Cancer Treatment Group (NCCTG) 0147 and the Cancer and Leukemia Group B 80405 trial [53]. The former is a phase III adjuvant study of oxaliplatin plus 5-fluorouracil and leucovorin with or without cetuximab in resected stage III CRC patients. The latter is a phase III study investigating the role of combined antibody therapy (cetuximab and bevacizumab) in combination with chemotherapy as first-line therapy for metastatic disease. Preliminary analysis of the NCCTG 0147 trial indicates that cetuximab plus FOLFOX does not produce a superior outcome compared with FOLFOX alone as adjuvant therapy for stage III CRC patients, regardless of *KRAS* status.

CLINICAL TRIALS IN *KRAS* MUTATED TUMORS AND FUTURE DIRECTIONS

As therapeutic options for patients with *KRAS* mutated tumors decrease, clinical trials dedicated to this population are essential. Currently, several early-phase pharmaceutical company-initiated trials are investigating novel drugs either alone or in combination with other chemotherapeutic or biologic agents [53]. As mentioned previously, an investigator-led trial from Denmark (NCT00827684) will study the potential benefit of the mTOR inhibitor temsirolimus in *KRAS* mutants [53]. Finally, a phase II National Cancer Institute-sponsored trial (NCT00343772) will investigate the effect of the oral multikinase inhibitor sorafenib in combination with cetuximab in the treatment of patients with both WT and mutant *KRAS* tumors [53]. Response will be correlated with *KRAS* and *BRAF* status, as well as other molecular characteristics. Emerging studies investigating the role of sorafenib in *BRAF* mutated tumors are also of interest in optimizing existing therapy for patients with WT *KRAS* tumors.

CONCLUSION

KRAS mutational status has definitively emerged as a predictive marker for response to anti-EGFR antibody therapy in CRC. The *KRAS* story is unique in that the scientific community and professional organizations embraced these data prior to the regulatory community; in this case, regulatory bodies were faced with an abundance of retrospective data but a lack of prospectively validated data. The FDA ultimately endorsed the predictive role of *KRAS*, however, based solely on rigorous retrospective, correlative research. The predictive role of *KRAS* was since demonstrated very recently in two prospective studies. Ongoing investigation of other markers that may similarly predict resistance to EGFR inhibitors may help to further identify patients with WT *KRAS* who might not derive benefit from these drugs. Limiting cetuximab and panitumumab to patients who will

truly benefit from these agents will improve the effectiveness and cost-effectiveness of CRC therapy. Ultimately, development of therapeutics that may bypass mutations in the EGFR pathway may provide therapeutic alternatives for patients with CRC.

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