

# Using Predictive Biomarkers to Select Patients With Advanced Colorectal Cancer for Treatment With Epidermal Growth Factor Receptor Antibodies

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As a result of the data emerging during the course of 2008, including presentations at the 44th Annual Meeting of the American Society of Oncology, it has generally been accepted that selection of patients with metastatic colorectal cancer (mCRC) for treatment with epidermal growth factor receptor (EGFR) antibodies—cetuximab or panitumumab—is reliant on the *KRAS* status of the tumor. For some time, *KRAS* had been suggested as a predictive marker for resistance to EGFR monoclonal antibodies, but Amado et al<sup>1</sup> were the first to publish conclusive data demonstrating the relationship between *KRAS* status and panitumumab efficacy in their analysis of tumor sections from participants in a randomized phase III trial comparing panitumumab with best supportive care. They found that both response to panitumumab monotherapy and improvement in progression-free survival (PFS) were confined to patients with *KRAS* wild-type (WT) mutations. *KRAS* mutations were detected in 43% of patients, none of whom responded to panitumumab.

Analyses of *KRAS* status and response to cetuximab have revealed similar results. For instance, in the first-line treatment of mCRC, a retrospective analysis of the impact of *KRAS* status on PFS and response rate on patients treated with folinic acid, fluorouracil, and irinotecan with or without cetuximab for mCRC within the Cetuximab Combined with Irinotecan in First-Line Therapy for Metastatic Colorectal Cancer trial identified *KRAS* mutations in 35.6% of *KRAS*-assessable patients.<sup>2</sup> For *KRAS* WT patients, both PFS (9.9 v 8.7 months; HR, 0.68;  $P = .017$ ) and response rate (59.3% v 43.2%;  $P = .0025$ ) were significantly improved by the addition of cetuximab to folinic acid, fluorouracil, and irinotecan. In contrast, for patients with *KRAS* mutations, there was no significant difference in either PFS (7.6 v 8.1 months; HR, 1.07;  $P = .47$ ) or response rate (40.2% v 36.2%;  $P = .46$ ) with the addition of cetuximab. For patients treated with first-line infused fluorouracil, folinic acid, and oxaliplatin with or without cetuximab in the Oxaliplatin and Cetuximab for First-Line Treatment of Metastatic Colorectal Cancer study, the improved response rate and PFS associated with cetuximab was also limited to those with *KRAS* WT tumors.<sup>3</sup> In fact, in this study, patients with mutated *KRAS* receiving cetuximab had poorer outcomes compared with those receiving infusional fluorouracil, leucovorin, and oxaliplatin alone.

Based on these results, it is now recommended that *KRAS* testing to exclude the presence of mutations should be performed when

considering either panitumumab or cetuximab in the treatment of advanced colorectal cancer.

Ligand-receptor activation of the EGFR at the cell surface results in homo- or heterodimerization of the receptors and triggers activation of downstream signaling pathways.<sup>4</sup> The incidence of *KRAS* mutations in colorectal tumors is approximately 35% to 45%. *KRAS* mutations can result in constitutive activation of the Ras-Raf-MAP-kinase pathway, one of the major EGFR downstream pathways, and therefore confer resistance to EGFR antibodies.<sup>5</sup> Nonmutated *KRAS* does not, however, guarantee benefit from treatment with EGFR monoclonal antibodies. Consequently, even with routine testing of *KRAS* status, a significant proportion of patients will be exposed to these drugs and their associated toxicity without deriving any benefit.

In this issue of *Journal of Clinical Oncology*, Di Nicolantonio et al<sup>6</sup> evaluate the role of *BRAF* mutations as prognostic or predictive factors for response to cetuximab or panitumumab and explore potential ways to circumvent inherent pathways of resistance. In this hypothesis-generating study, a retrospective analysis of 113 patients treated with either cetuximab or panitumumab was conducted. Additionally, a cellular analysis of the effect of the *BRAF* V600E allele on response to cetuximab or panitumumab was performed.

The presence of *KRAS* mutations in this population was 30%. As expected, *KRAS* mutations were associated with a lack of response to EGFR antibodies ( $P = .011$ ) and shorter PFS ( $P = .0275$ ) compared with *KRAS* WT tumors. Twenty-eight percent of *KRAS* WT patients responded to either cetuximab (alone or in combination with irinotecan) or panitumumab. *BRAF* mutations (*BRAF* V600E allele) were identified in 11 patients (10% of the population evaluated; 14% of *KRAS* WT patients). *BRAF* and *KRAS* mutations were mutually exclusive, as observed in previous studies.<sup>7</sup> Supporting the authors' hypothesis that in *KRAS* WT tumors, *BRAF* mutations could have predictive value, none of the 11 *BRAF*-mutated tumors responded to treatment. Similarly, in the laboratory, colorectal cancer cell lines carrying the *BRAF* V600E allele were highly refractory to cetuximab and panitumumab. Conversely, all 22 patients who responded to *KRAS* WT also had *BRAF* WT. *BRAF*-mutated tumors were also associated with shorter PFS and overall survival irrespective of *KRAS* status ( $P = .0107$  and  $P < .0001$ , respectively), suggesting a potential role of *BRAF* as a prognostic biomarker. Interestingly, targeting *BRAF*-mutated cell lines with the combination of cetuximab and sorafenib resulted in much higher response rates than those observed

with exposure to either agent alone—an effect that could be exploited clinically if validated.

In summary, these are interesting results that may provide some insight into the mechanisms underlying inherent resistance to EGFR monoclonal antibodies and possible therapeutic approaches to induce sensitivity. The results are somewhat limited by the retrospective nature of the study, the relatively small numbers, and the treatment of patients outside the context of a randomized clinical trial. In particular, the lack of a non-EGFR antibody treatment control arm makes it difficult to form a conclusive statement on the prognostic impact of *BRAF* mutations. However, the reported response rates are consistent with currently available literature.<sup>8-10</sup>

Clearly, *KRAS* or *BRAF* mutations are not the only factors influencing response to EGFR monoclonal antibodies. In this study, two patients (6%) with *KRAS* mutations responded to treatment; however, it is possible that these patients may have received a combination of irinotecan and cetuximab and may not have been truly irinotecan refractory. On the other hand, 41% of patients had neither *KRAS* nor *BRAF* mutations and yet still did not respond to EGFR antibody-containing therapy. Ongoing identification and evaluation of other predictive biomarkers are imperative to improve the selection of candidates for treatment.

The Bowel Oncology with Cetuximab Antibody (BOND) study,<sup>8</sup> comparing irinotecan plus cetuximab with cetuximab alone in irinotecan-refractory patients, was pivotal in establishing the role of cetuximab as a valid therapy, with response rates of 22.9% in the combination-therapy group and 10.8% in the monotherapy group. Entry criteria to BOND mandated EGFR expression by immunohistochemistry (IHC) in either the primary tumor or at least one metastatic lesion. From the results of BOND, it became apparent that the degree of EGFR expression (either by percentage of staining cells or staining intensity) did not correlate with response. Subsequent studies have demonstrated a response to cetuximab in some patients who do not express the EGFR by IHC.<sup>11,12</sup> A possible explanation for this observation is that given its inherent subjectiveness, IHC is a suboptimal method for assessing EGFR status. It is, however, plausible that the percentage of active EGFRs is more important when predicting EGFR antibody sensitivity. Phosphorylated EGFR is a surrogate marker of EGFR activity and has been associated with response to gefitinib in both pulmonary adenocarcinoma and colorectal cancer cell lines.<sup>13,14</sup> Further evaluation of the role of phosphorylated EGFR as a predictive marker for response to cetuximab and panitumumab is warranted.

Another potential biomarker for response to EGFR antibodies is the EGFR-associated rash. The severity of the characteristic maculopapular rash associated with EGFR blockade has been correlated with response to cetuximab and improved survival in BOND and other studies.<sup>8</sup> Tejpar et al<sup>15</sup> have assessed the impact of *KRAS* status on efficacy within the Evaluation of Various Erbitux Regimens by Means of Skin Tumor Biopsies trial, which was designed to investigate the role of cetuximab-dose escalation according to severity of skin rash. Patients randomly assigned to the dose-escalation arm achieved higher response rates and PFS compared with those acting as controls. Subsequent analysis of *KRAS* status indicates that dose escalation does not improve response to cetuximab in patients with *KRAS* mutations; however, for patients with *KRAS* WT, severity of skin toxicity correlates with a greater PFS, suggesting that skin toxicity and *KRAS* status are independent predictors of efficacy.<sup>16</sup> Dose escalation to induce

response in nonresponding patients with *KRAS* WT may therefore be a valid therapeutic maneuver. It is possible that dose escalation maximizes blockade of upregulated active EGFRs. Alternatively, soluble EGFRs, which have been identified in breast and ovarian cancers, may form complexes with EGFR antibodies, thereby reducing available circulating EGFR antibody levels. Hence, increasing cetuximab or panitumumab dose may overcome this phenomenon.<sup>17,18</sup>

Increased EGFR gene copy number (GCN) has also been proposed as a potential marker of EGFR activity, with improved response observed in at least two studies.<sup>19,20</sup> These results, however, were not reproduced in other studies, indicating that further clarification of the role of EGFR GCN in mCRC is required.<sup>12,21</sup> The differing methods of assessing EGFR GCN (fluorescent or chromosomal in situ hybridization and quantitative polymerase chain reaction) may partially explain the discrepant results, and highlight the need for standardized and easily reproducible methods of assessing biomarkers.

High mRNA levels of the EGFR ligands ephrins and amphiregulin have been associated with increased responsiveness to cetuximab, and it is thought that tumors with these characteristics may be particularly dependent on the EGFR for growth.<sup>21</sup> Improved outcome has been reported in patients with *KRAS* WT with high ephrins and amphiregulin expression who were treated with cetuximab.<sup>22</sup> Additional data suggest that other EGFR downstream pathways such as the *PI3K/PTEN/AKT/mTOR* and *JAK/STAT* pathways are also important when considering mechanisms of EGFR antibody resistance. *PTEN* mutations occur in approximately 20% of sporadic CRC.<sup>23</sup> At least three studies have correlated either loss of *PTEN* gene function/expression (characterized by IHC or polymerase chain reaction [PCR]) or mutations in *PI3KCA* with nonresponsiveness to cetuximab.<sup>24-26</sup> These findings are consistent with *PTEN*'s known role as a tumor suppressor via negative regulation of *PI3K*, and validate results previously observed in cell line studies.<sup>27</sup>

Although prospective validation of these and other biomarkers within the context of randomized clinical trials is required before adoption into routine clinical practice, these studies emphasize the importance of understanding the mechanisms of oncogenesis at a molecular level. It seems likely that additional testing of patients with *KRAS* WT for *BRAF* mutations will further enrich the population that should be considered for treatment with EGFR antibodies. As demonstrated by the study by Di Nicolantonio et al<sup>6</sup> and the Evaluation of Various Erbitux Regimens by Means of Skin Tumor Biopsies trial, identifying the possible mechanisms of resistance to EGFR antibodies may also allow development of therapeutic strategies to overcome resistance in different subsets of patients. Ideally, biomarkers should provide a definitive answer to the question of whether a response to EGFR inhibitors is expected, and should not be prone to subjective interpretation. A further consideration is whether biomarkers represent events occurring early or late in the carcinogenesis pathway, with the latter potentially requiring rebiopsy of metastatic lesions if there is discordance with the primary tumor. With ongoing collaborative efforts between clinicians, scientists, and pathologists, tailoring treatment to individual patients is slowly but surely becoming a reality.

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