

## ***KRAS* Mutation in Colon Cancer: A Marker of Resistance to EGFR-I Therapy**

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### **ABSTRACT**

**Introduction and Design.** The introduction of the epidermal growth factor receptor inhibitors (EGFR-I) has increased the treatment options available for patients with metastatic colorectal cancer (mCRC). Two EGFR-I agents currently approved for the treatment of mCRC are the fully human monoclonal antibody panitumumab and the mouse-human chimeric monoclonal antibody cetuximab. While these agents have demonstrated activity across multiple lines of therapy, early studies suggested that clinical benefit was confined to a subset of patients treated. Mutation of the *KRAS* oncogene has emerged as a powerful negative predictive biomarker to identify patients with mCRC who do not benefit from EGFR-I therapy. Multiple retrospective analyses have demonstrated that clinical benefit from treatment with EGFR-I is limited to patients with tumors harboring the wild-type *KRAS* gene. In this review, the *KRAS* pathway and studies evaluating *KRAS* as a prognostic marker in CRC are discussed along with advances in *KRAS* gene mutation testing. Clinical trials evaluating the role of *KRAS* status in response to EGFR-I monotherapy or in combination with chemotherapy are also highlighted along with ongoing studies evaluating the role of EGFR-I treatment on curative resections rates.

**Results and Conclusion.** Future studies investigating EGFR-I therapy in mCRC should incorporate *KRAS* mutation testing into the study protocol in order to more accurately determine the patient population that will obtain clinical benefit from these novel agents.

Colorectal cancer (CRC) remains the fourth leading cause of cancer diagnosis and the second leading cause of cancer-related deaths in the United States.<sup>1</sup> Treatment of patients with metastatic colorectal cancer (mCRC) has dramatically changed over the last decade. A marked advance in the treatment of patients with mCRC is represented by the monoclonal antibody epidermal growth factor receptor inhibitors (EGFR-I), including the fully human monoclonal antibody panitumumab and the mouse-human chimeric monoclonal antibody cetuximab. The small molecule inhibitors of the EGFR tyrosine kinase domain, erlotinib and gefitinib, have demonstrated activity in non-small-cell lung cancer but have not demonstrated a clinically important benefit in patients with mCRC.<sup>2,3</sup> Both of the monoclonal antibody EGFR-I are approved for use in patients with mCRC as monotherapy, and cetuximab is also approved in combination with irinotecan.<sup>4,5</sup> Studies with EGFR-I demonstrate that a select group of patients with mCRC exhibit clinical benefit, with response rates of approximately 10% observed across several large EGFR-I monotherapy clinical trials.<sup>6–8</sup> Despite a lack of myelosuppression, EGFR-I therapy is associated with marked adverse effects, including skin rash, diarrhea, and hypomagnesemia.<sup>9,10</sup> To improve quality of life and patient clinical outcomes, the selection of patients who would benefit from EGFR-I is of paramount importance, and testing of *KRAS* may help to improve selection of these patients.

K-ras (OMIM 190070) is a member of the Ras family of small G proteins involved in intracellular signaling.<sup>11</sup> Activating mutations in *KRAS* results in the constitutive activation of downstream signaling pathways and confers resistance to inhibition of cell surface receptor tyrosine kinases, including EGFR.<sup>12</sup>

Several studies have evaluated the role of *KRAS* mutation as both a prognostic and predictive marker.<sup>13–27</sup> Prognostic markers provide information on the outcome of the patient irrespective of the therapeutic intervention, while predictive markers are specific to the therapy

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**TABLE 1** Incidence and rate of response of *KRAS* mutation within selected studies evaluating *KRAS* as a predictive biomarker to epidermal growth factor receptor inhibitor therapy

Treatment	<i>N</i>	<i>KRAS</i> WT <i>N</i> (%)	<i>KRAS</i> MT <i>N</i> (%)	RR% <i>KRAS</i> WT (%)	RR% <i>KRAS</i> MT (%)	References
Cetuximab + irinotecan, cetuximab + FOLFIRI, cetuximab	30	17 (57%)	13 (43%)	65	0	Lievre et al. <sup>13</sup>
Panitumumab, cetuximab + irinotecan, cetuximab	48	32 (67%)	16 (33%)	31	6	Benvenuti et al. <sup>14</sup>
Cetuximab, cetuximab + irinotecan	108	66 (61%)	42 (39%)	41	0	De Roock et al. <sup>15</sup>
Cetuximab + irinotecan, cetuximab, cetuximab + oxaliplatin	81	49 (60%)	32 (40%)	27	6.3	Finocchiaro et al. <sup>16</sup>
Cetuximab + irinotecan, cetuximab + FOLFIRI, cetuximab	89	65 (73%)	24 (27%)	40	0	Lievre et al. <sup>17</sup>
Cetuximab	80	50 (62%)	30 (38%)	48 <sup>a</sup>	10 <sup>a</sup>	Khambata-Ford et al. <sup>18</sup>
Panitumumab	62	38 (61%)	24 (39%)	11	0	Freeman et al. <sup>19</sup>

WT wild type, RR% response rate, CR complete response, PR partial response, SD stable disease, MT mutant

<sup>a</sup> Reported as percentage of disease control (CR + PR + SD)

administered to the patient. *KRAS* mutation occurs early in CRC carcinogenesis and was observed in 27–43% of patients with CRC (Table 1).<sup>13–19</sup> Several older studies suggest that *KRAS* mutation is prognostic in CRC patients.<sup>20,21</sup> However, recent studies continue to question the prognostic value of *KRAS* in mCRC.<sup>22,23</sup>

Biomarker analysis from several recent studies demonstrated that patients with *KRAS* mutated tumors are resistant to monotherapy with cetuximab or panitumumab.<sup>14,23,24</sup> The additional benefit of EGFR-I to chemotherapy is limited to patients with wild-type (WT) *KRAS* mCRC.<sup>25–27</sup> However, the optimal biologic agent (bevacizumab or EGFR-I) to be combined with chemotherapy for the first- or second-line treatment of patients with WT *KRAS* mCRC remains to be determined.

Here, we review recent studies involving EGFR-I in advanced CRC with particular attention to incidence, prognostic value, and predictive significance of the *KRAS* mutation in CRC patients.

## KRAS MUTATION IN CRC

The *KRAS* oncogene encodes the human cellular homolog of the transforming gene Kirsten rat sarcoma-2 virus.<sup>11</sup> The K-ras protein is a self-inactivating signal transducer. K-ras cycles between a guanosine diphosphate (GDP) bound (“off” state) to guanosine triphosphate (GTP) bound (“on” state) in response to receptor activation. When receptor tyrosine kinases, such as EGFR, bind ligands (e.g., epidermal growth factor, transforming growth factor- $\alpha$ ), the receptor dimerizes and undergoes a conformational change that results in phosphorylation of tyrosine residues, which leads to activation of signaling pathways that include the Ras-Raf-Mek kinase pathway. This response is usually transient as a

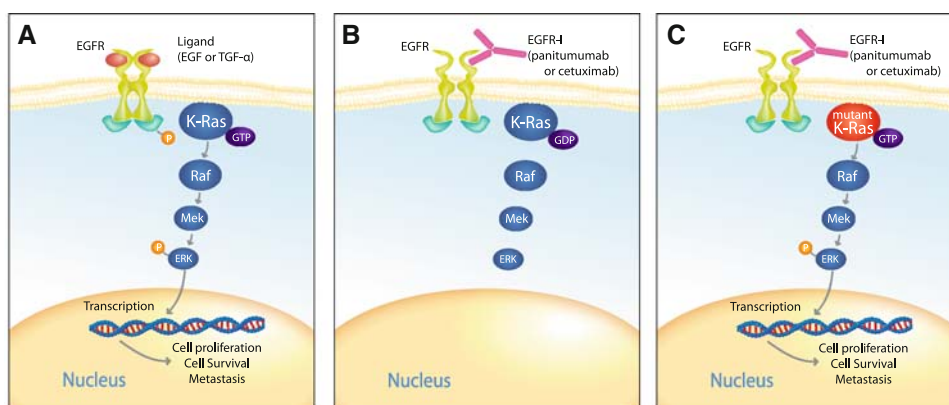
result of the intrinsic GTPase activity of K-ras.<sup>12</sup> However, *KRAS* activating mutations abolish this intrinsic GTPase activity, resulting in constitutively active K-ras proteins that activate downstream signaling pathways and can lead to oncogenesis (Fig. 1).<sup>12</sup>

Activating *KRAS* mutations are among the most common oncogenic alterations in cancer, occurring early in CRC carcinogenesis.<sup>28</sup> In one study, 58% of colonic adenomas of >1 cm carried the *KRAS* mutation.<sup>29</sup> The importance of *KRAS* to colorectal tumorigenesis is underscored by the finding that colon cancer cells in which a mutated *KRAS* gene has been removed or replaced lose their ability to form tumors in mice.<sup>30</sup> The incidence rate of *KRAS* mutations is fairly consistent across several studies, and a similar incidence rate has been reported both in early-stage and metastatic disease.<sup>21,31–33</sup>

## TESTING FOR KRAS MUTATIONS

Currently, several methods are used to detect *KRAS* mutations within tumors. One method that is generally used for testing *KRAS* mutations is an allele-specific polymerase chain reaction on DNA from tumor sections, typically obtained from a formalin-fixed, paraffin-embedded block. This assay uses an allele-specific primer extension that can detect the most common *KRAS* mutations located within codons 12 and 13 of the gene.

Several commercial assays are available, and currently there is no standardization. Recently, a comparability study of four commercially available *KRAS* tests was presented at the annual meeting of the American Association for Cancer Research.<sup>34</sup> This analysis suggested that assays by allele-specific polymerase chain reaction or direct sequencing accurately detected the presence of mutations within the



**FIG. 1** K-ras mutation governs the effects of inhibition of epidermal growth factor receptor (EGFR) on the Ras/Raf/Mek signaling pathway. **a** The ligands epidermal growth factor (EGF) or transforming growth factor (TGF)- $\alpha$  activate EGFR signaling leading to phosphorylation of the tyrosine kinase domain. K-Ras adopts a guanosine triphosphate (GTP) bound conformation and through a series of phosphorelays activates ERK. Activation of ERK leads to transcription of genes associated with cell proliferation, survival, and

metastasis. **b** Inhibition of EGFR by the EGFR-I panitumumab or cetuximab leads to K-ras becoming guanosine diphosphate (GDP) bound, which inhibits downstream signaling. **c** When K-ras is mutated, it adopts a constitutively GTP bound conformation leading to activation of the Ras/Raf/Mek pathway in the presence of inhibition of EGFR. Adapted with permission from Khambata-Ford et al.<sup>18</sup>

**TABLE 2** Comparison of four commercially available *KRAS* mutation tests to the expected standard determined by direct sequencing conducted by an internal core laboratory<sup>34</sup>

Mutation	Allele-specific PCR (DxS/Histogenex)	Direct sequencing (Gentris)	Allele-specific hybridization (Invitek)	Allele-specific PCR extension (Genzyme)
<i>KRAS</i> WT detected/expected	20/21	18/21	3/21	15/21
<i>KRAS</i> mutations detected/expected	20/19	14/19	24/19	20/19
Inconclusive <sup>a</sup>	0	8	13	5

PCR polymerase chain reaction, WT wild type

<sup>a</sup> *KRAS* tests with inconclusive, failed, or mixed results

*KRAS* gene in a high percentage of patient tumor samples when compared to direct sequencing conducted by an internal laboratory. However, an assay that used direct hybridization technology failed to detect the presence of a mutation with the same accuracy (Table 2).<sup>34</sup> Recently, the American Society of Clinical Oncology (ASCO) released a provisional opinion identifying allele-specific polymerase chain reaction and direct sequencing as acceptable methods for testing of *KRAS* mutations.<sup>35</sup> In addition, a group of physicians from the European Union have proposed guidelines for a European Union quality assurance program to establish consistency in testing practices across Europe.<sup>36</sup> When testing for the presence of a *KRAS* mutation from patient tumor samples, clinicians should ensure the validity of the assay through the use of control DNA with known mutation status.

### ***KRAS* MUTATION AS A PROGNOSTIC MARKER**

Studies evaluating the role of *KRAS* mutation as a prognostic marker in patients with CRC have somewhat

conflicting results. A study of patients with stage II/III CRC demonstrated that *KRAS* mutant tumoral status was associated with an increase in the rate of recurrence of their disease (71%) compared to WT (25%).<sup>20</sup> Another study demonstrated that the prognostic value of *KRAS* mutation was limited to patients with stage I/II CRC.<sup>21</sup> Several other studies have demonstrated data in support of *KRAS* mutation as a negative prognostic marker, while others have concluded otherwise.<sup>31,32,37–39</sup>

In patients with mCRC, the prognostic value of *KRAS* also presents conflicting results. Patients with advanced disease receiving fluorouracil demonstrated no difference in survival on the basis of *KRAS* status.<sup>22</sup> In addition, the NCIC CO.17 trial of patients with chemorefractory mCRC treated with cetuximab versus best supportive care (BSC) demonstrated no survival differences among patients with WT *KRAS* or mutant tumors receiving BSC.<sup>23</sup> In contrast, the updated *KRAS* analysis of the pivotal bevacizumab with or without chemotherapy (IFL) study demonstrated lower median overall survival for mutant tumor *KRAS* patients versus WT in both treatment arms (IFL:

17.6 months WT vs. 13.6 months mutant; IFL with bevacizumab: 27.7 months WT vs. 19.9 months mutant), although statistical models were not powered to detect the significance of these differences.<sup>40</sup> Therefore, the prognostic significance of *KRAS* in patients with CRC is still inconclusive. The most likely explanation for the disparity in the results is the heterogeneity of the patient population. Future studies should attempt to stratify patient groups by stage of disease, primary tumor site, and presence of other biomarkers.

### KRAS MUTATION PREDICTS RESISTANCE TO EGFR-I THERAPY

Early small retrospective studies correlated activating mutation of *KRAS* with resistance or lack of response to EGFR-I in patients with mCRC, establishing the proof of concept for further investigation of this biomarker.<sup>13–19</sup> In these studies, patients were treated with panitumumab or cetuximab monotherapy, or cetuximab in combination with irinotecan or other chemotherapeutic agents (Table 1).<sup>13–19</sup> Mutant *KRAS* status in these patients was associated with a lack of response to EGFR-I-based therapy. Most patients with mutated *KRAS* tumors did not have an objective response when treated with EGFR-I-based therapy (Table 1). Although the role of *KRAS* mutation as a negative predictive marker of response to therapy was demonstrated in these studies, definitive conclusions could not be made as a result of the small patient numbers and differences in treatment.

The first large study to analyze the effect of *KRAS* mutational status with efficacy with EGFR-I monotherapy was conducted with patient tumor samples from the pivotal panitumumab registrational trial.<sup>24</sup> In this randomized phase 3 trial, patients with chemorefractory mCRC were randomized to panitumumab or BSC, allowing patients to cross over from BSC to panitumumab at disease progression.<sup>8</sup> *KRAS* status was determined in 427 (92%) of 463 patients (208 panitumumab and 219 BSC) with *KRAS*

mutations identified in 184 (43%) of 427 patients (84 [40%] in the panitumumab arm).<sup>24</sup> In the subset of patients with WT *KRAS* tumors, progression-free survival (PFS) was improved with panitumumab versus BSC treatment (Table 3). Median PFS time was 2.8 months for panitumumab vs. 1.7 months for BSC in the WT group (hazard ratio [HR] .45,  $P < .0001$ ). No benefit was seen regardless of treatment in patients with mutant *KRAS* tumors. Response rates to panitumumab were 17 and 0% for the WT and mutant groups, respectively. In patients randomized to BSC who subsequently received panitumumab in the cross over study, PFS was longer in patients with WT *KRAS* tumors (3.8 months WT vs. 1.8 months mutant (HR .32; 95% confidence interval [95% CI] 0.22–0.46). The results of these analyses demonstrated that response to panitumumab was confined to patients with WT *KRAS* tumors.<sup>24</sup>

Similar findings have been demonstrated in a large randomized phase 3 trial of patients treated with cetuximab monotherapy.<sup>23</sup> In this study, 572 patients with chemorefractory mCRC were randomized to cetuximab versus BSC. The crossover of BSC patients to the cetuximab arm of the trial was not mandated by the study protocol.<sup>7</sup> Tumor specimens were available for *KRAS* mutational testing from 394 patients (69%) with mutations detected in 42% of these patients. Among patients treated with cetuximab, the objective response rate for WT *KRAS* patients was 12.8%, compared with a 1.2% response rate for patients in the *KRAS* mutant group. Cetuximab monotherapy was associated with significant improvements in PFS (3.7 vs. 1.9 months,  $P < .001$ ) and overall survival in WT *KRAS* patients treated with cetuximab (9.5 months for cetuximab vs. 4.8 months for BSC,  $P < .001$ ) (Table 3). However, increased overall survival was not observed in patients with *KRAS*-mutated tumors (4.5 months for cetuximab vs. 4.6 months for BSC,  $P = .89$ ).

The data from these studies strongly support the hypothesis that the activating mutation in *KRAS* confers resistance to EGFR-I. The clinical benefit from single-

**TABLE 3** Response rate and progression-free survival of patients treated in phase 3 epidermal growth factor receptor inhibitor monotherapy trials by *KRAS* mutational status<sup>23,24</sup>

<i>KRAS</i>	Panitumumab	BSC	HR ( <i>P</i> )	Cetuximab	BSC	HR ( <i>P</i> )
<b>WT <i>KRAS</i></b>						
Response rate	17%	0%		12.8%	0%	
Median PFS	2.8 mo <sup>a</sup>	1.7 mo <sup>a</sup>	.45 (<.0001)	3.7 mo	1.9 mo	.40 (<.001)
<b>Mutant <i>KRAS</i></b>						
Response rate	0%	0%		1.2%	0%	
Median PFS	1.7 mo <sup>a</sup>	1.7 mo <sup>a</sup>	.99 (NR)	1.8 mo	1.8 mo	.99 (.96)

NR not reported, *PFS* progression free survival, *BSC* best supportive care, *HR* hazard ratio for progression-free survival

<sup>a</sup> Reported as weeks in the original publication

agent panitumumab and cetuximab is restricted to patients with WT *KRAS*. In WT *KRAS* patients, EGFR-I treatment is associated with marked objective response, and improvements in PFS and overall survival over BSC in patients with chemorefractory mCRC.

### **BENEFIT FROM THE ADDITION OF EGFR-I TO CHEMOTHERAPY IS LIMITED TO WT *KRAS* PATIENTS**

Several recent studies have evaluated the addition of cetuximab or panitumumab to standard chemotherapy in first- or second-line setting in patients with mCRC. Data from these studies have demonstrated an association between *KRAS* mutational status and response to the addition of EGFR-I to standard chemotherapy.

Two studies presented at the 2009 ASCO annual meeting examined the safety and efficacy of panitumumab in combination with irinotecan-based chemotherapy in patients with mCRC whose disease previously failed to respond to oxaliplatin-based therapy. In the PRECEPT trial, the role of *KRAS* is being prospectively evaluated in patients with mCRC treated with panitumumab with FOLFIRI; the STEPP study also evaluated the efficacy of panitumumab in combination with FOLFIRI or irinotecan with *KRAS* mutational status available for 92% of patients.<sup>25,41</sup> In both of these studies, clinical end points for efficacy of panitumumab with chemotherapy were in favor of patients with WT *KRAS* tumors.<sup>25,41</sup>

A more favorable efficacy profile was reported among patients with WT *KRAS* mCRC receiving cetuximab in combination with FOLFOX in a randomized phase 2 trial evaluating FOLFOX with or without cetuximab as first-line treatment (OPUS).<sup>26</sup> Efficacy results of the WT *KRAS* population demonstrated that response rate greatly increased when cetuximab was added to FOLFOX (61 vs. 37%,  $P = .011$ ). Conversely, patients with mutant *KRAS* had a nonsignificant decrease in response rate when cetuximab was added to FOLFOX (33 vs. 49%,  $P = .106$ ). These data suggest that clinical benefit from addition of cetuximab to FOLFOX is limited to patients with WT *KRAS*.

In the CRYSTAL trial, previously untreated patients with mCRC were randomized to FOLFIRI with or without cetuximab.<sup>27</sup> Of the 1198 patients analyzed in the original intention-to-treat population, 540 (45%) were included in the *KRAS* analysis and 36% had a *KRAS* mutation. The addition of cetuximab to FOLFIRI in patients with WT *KRAS* tumors resulted in a nonsignificant increase in median PFS compared to patients receiving FOLFIRI alone (9.9 vs. 8.7 months,  $P = .07$ ). In contrast, the median PFS of mutant tumor *KRAS* patients receiving cetuximab with

FOLFIRI was lower (7.6 months) compared with patients receiving FOLFIRI alone (8.1 months, HR 1.07, 95% CI 0.71–1.61). A nonsignificant increase in overall survival was observed in WT *KRAS* patients treated with cetuximab with FOLFIRI compared to patients treated with FOLFIRI alone (24.9 vs. 21 months,  $P = .44$ ).<sup>27</sup> These data demonstrate that the benefit observed from the addition of cetuximab to chemotherapy is limited to patients with WT *KRAS* tumors; however, future studies will be needed to determine the optimal clinical settings for the use of cetuximab in combination with FOLFIRI in the first-line treatment of mCRC.

An important finding from the CRYSTAL trial was the observation that the surgical resection rate of metastases was higher in patients treated with cetuximab with FOLFIRI compared to patients treated with FOLFIRI alone (7.0 vs. 3.7%).<sup>27</sup> In addition, the R0 resection rate with curative intent was statistically higher in favor of patients receiving cetuximab (4.8 vs. 1.7%; odds ratio for cetuximab with FOLFIRI group, 3.02; 95% CI, 1.45–6.27;  $P = .002$ ).<sup>27</sup> However, the relationship of these findings from the CRYSTAL trial to *KRAS* status of the tumor is currently unknown.

Recently, at the 2009 Gastrointestinal Cancers Symposium, Folprecht et al. presented preliminary data from a randomized multicenter study of cetuximab with FOLFOX or FOLFIRI in neoadjuvant treatment of nonresectable colorectal liver metastasis (CELIM study).<sup>42</sup> A confirmed overall response rate of 70% was observed in patients with WT *KRAS* tumors treated with FOLFOX or FOLFIRI with cetuximab.<sup>42</sup> Among all patients with disease that was initially deemed nonresectable, 25% were classified as resectable after treatment. Overall, 34% of patients enrolled onto the CELIM study underwent R0 resection and demonstrated no evidence of residual disease.<sup>42</sup> Perioperative morbidity and mortality were comparable to historical data. These preliminary data are encouraging and warrant further studies of evaluating EGFR-I in combination with chemotherapy as a neoadjuvant therapy in patients with *KRAS* WT tumors with liver metastasis.

Two current phase 3 studies are evaluating the influence of *KRAS* on response to panitumumab with chemotherapy in patients with mCRC. The PRIME study is evaluating FOLFOX with or without panitumumab in the first-line setting, while the 20050181 study is evaluating FOLFIRI with or without panitumumab for second-line treatment.<sup>43,44</sup> Tumor sample availability was required for enrollment onto these studies, and *KRAS* status will be incorporated into the primary analysis to test the role of *KRAS* in the combination therapy setting. Future trials seeking to evaluate the role of a biomarker such as *KRAS* may benefit from the incorporation of biomarker evaluation into the study design.

## OPTIMAL BIOLOGICS IN WT *KRAS* IS STILL UNKNOWN

The studies mentioned above support the beneficial effect of the addition of EGFR-I to standard chemotherapy in patients with WT *KRAS* mCRC. However, the optimal biologics to combine with chemotherapy in first- and second-line setting remains unclear.

Retrospective analysis of the pivotal trial of bevacizumab with IFL demonstrated no statistically significant correlation between *KRAS* mutation and the benefit from bevacizumab.<sup>40</sup> The median PFS for chemotherapy alone versus chemotherapy with bevacizumab was 7.4 vs. 13.5 months (HR .44;  $P < .0001$ ) in WT *KRAS* and 5.5 and 9.3 months (HR .41;  $P < .0008$ ) in *KRAS* mutant groups.

Two recent trials have evaluated the safety and efficacy of the addition of EGFR-I to standard chemotherapy and bevacizumab in treatment-naïve patients with mCRC. The first study to evaluate this combination was the PACCE trial.<sup>45</sup> In this phase 3 study, patients were randomized to receive chemotherapy and bevacizumab with or without panitumumab. The chemotherapeutic regimen was either FOLFOX or FOLFIRI, based on the choice of the investigator. Efficacy results demonstrated that patients with WT or mutant *KRAS* tumors experienced a decrease in PFS when panitumumab was added to FOLFOX with bevacizumab versus FOLFOX with bevacizumab alone (Table 4). Similar results were demonstrated in patients treated in the FOLFIRI cohort (Table 4).<sup>45</sup>

The CAIRO2 trial evaluated the combination of capecitabine, oxaliplatin, and bevacizumab, with or without cetuximab.<sup>46</sup> For the total patient group, the addition of cetuximab was associated with a statistically significant decrease in median PFS and increased grade 3/4 adverse events. In patients with WT *KRAS*, there was no statistically significant change in median PFS by the addition of cetuximab (10.6 vs. 10.5 months,  $P = .3$ ) (Table 4). However, patients with mutant *KRAS* tumors treated with cetuximab with chemotherapy and bevacizumab had a statistically significant decrease in median PFS compared to mutant tumor *KRAS* patients not treated with a cetuximab-containing regimen (12.5 vs. 8.1 months,  $P = .003$ ) (Table 4). The results of the PACCE and CAIRO2 studies demonstrate that the addition of EGFR-I to chemotherapy and bevacizumab for first line treatment of mCRC does not improve clinical outcomes for patients with mCRC, regardless of *KRAS* mutational status, and may decrease clinical outcome. Furthermore, these data strongly suggest a class effect of EGFR-I when used in combination with bevacizumab and fluoropyrimidine-based chemotherapy.

Two ongoing studies will continue to evaluate the optimal biologics to combine with chemotherapy in patients with WT *KRAS* mCRC. In the CALGB 80405 study, patients will be randomized to chemotherapy with bevacizumab alone or cetuximab alone, or in combination in the first-line setting.<sup>47</sup> This study has been amended to include only patients with WT *KRAS* tumors. In the phase 2 SPIRITT study, patients with WT *KRAS* mCRC whose

**TABLE 4** Median progression-free survival of patients treated in trials evaluating the addition of epidermal growth factor receptor inhibitor therapy to fluoropyrimidine-based regimens in combination with bevacizumab

<i>KRAS</i>	PACCE oxaliplatin cohort <sup>a</sup>		
	FOLFOX + bevacizumab (months)	Panitumumab + FOLFOX + bevacizumab (months)	HR (95% CI)
WT <i>KRAS</i>	11.5	9.8	1.36 (1.04–1.77)
<i>KRAS</i> MT	11.0	10.4	1.25 (0.91–1.71)
<i>KRAS</i>	PACCE irinotecan cohort <sup>a</sup>		
	FOLFIRI + bevacizumab (months)	Panitumumab + FOLFIRI + bevacizumab (months)	HR (95% CI)
WT <i>KRAS</i>	12.5	10.0	1.50 (0.82–2.76)
<i>KRAS</i> MT	11.9	8.3	1.19 (0.65–2.21)
<i>KRAS</i>	CAIRO2 <sup>b</sup>		
	CAPEOX + bevacizumab (months)	Cetuximab + CAPEOX + bevacizumab (months)	<i>P</i> value
WT <i>KRAS</i>	10.6	10.5	.30
<i>KRAS</i> MT	12.5	8.1	.003

WT wild type, HR hazard ratio for progression-free survival, 95% CI 95% confidence interval, MT mutant

<sup>a</sup> Hecht et al.<sup>45</sup>

<sup>b</sup> Tol et al.<sup>46</sup>

disease progressed while receiving oxaliplatin-based chemotherapy and bevacizumab will be randomized to FOLFIRI with either bevacizumab or panitumumab.<sup>48</sup> These studies will provide further information about the optimal biologic to combine with standard chemotherapy in patients with WT *KRAS* mCRC.

### FUTURE DIRECTIONS IN BIOMARKER RESEARCH TO EGFR-I-BASED THERAPY

Mutation of the *KRAS* gene has emerged as a powerful biomarker of resistance to EGFR-I-based therapy. Although most studies focus on mutations within codons 12 and 13 of the *KRAS* gene, a recent analysis demonstrated that mutations within codons 61 and 146 also confer resistance to EGFR-based therapy.<sup>49</sup> Recent studies have demonstrated that in addition to *KRAS*, other biomarkers including *BRAF* (OMIM 164757) and *PIK3CA* (OMIM 171834) can increase the predictive ability of response to EGFR-I in patients with WT *KRAS* tumoral status.<sup>49–51</sup> However, data from another study suggest that *PIK3CA* does not predict for response to EGFR-I therapy, and further studies will be needed to validate *PIK3CA* as a predictive biomarker.<sup>52</sup> In addition, a retrospective analysis suggested that patients treated on the NCIC CO.17 clinical trial with WT *KRAS* tumors and high mRNA expression of the EGFR ligand epiregulin (OMIM 602061) may derive the greatest benefit from cetuximab therapy.<sup>53</sup> Continued evaluation of these biomarkers and others will allow for a personalized approach for the treatment of patients with mCRC.

### CONCLUSIONS

The desired goal in cancer therapy is to individualize treatment according to the underlying predictive factors to minimize the unnecessary adverse effects and complications from cancer treatment. Recent data on the presence of a *KRAS* mutation and lack of response to EGFR-I represent an important step toward achieving that goal. Great progress has been made in the treatment of patients with mCRC over the last 10 years. The availability of newer chemotherapy and biologics has increased treatment options for patients and clinicians.

The current data strongly support the hypothesis that patients with *KRAS* mutation with mCRC have disease resistant to EGFR-I therapy. The beneficial effects of EGFR-I monotherapy or in combination with chemotherapy are limited to patients with WT *KRAS* tumors. Preliminary data suggest that EGFR-I in combination with chemotherapy can result in improvements in tumor responses and can potentially lead to downstaging of patients with nonresectable *KRAS* WT liver metastases.

Testing for *KRAS* mutation should be strongly considered in patients with mCRC before the initiation of any EGFR-I. The current data do not support the addition of anti-EGFR monoclonal antibodies to standard chemotherapy with bevacizumab in the first-line setting. Ongoing trials will address the optimal biologics to combine with chemotherapy in WT *KRAS* patients.

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**NOTE ADDED IN PROOF** During the review of this paper, the results of the PRIME and 20050181 phase 3 clinical trials discussed above were presented at the 2009 Joint ECCO 15—ESMO 34th Multidisciplinary Congress in Berlin, Germany. The results of both of these studies demonstrated significant increases in median PFS of patients with WT *KRAS* mCRC who received chemotherapy with panitumumab compared with patients receiving chemotherapy alone. (Peeters M, et al. *European Journal of Cancer Supplements*, Vol. 7, No 3, September 2009, Page 9, Abstract 14LBA; Douillard J, et al. *European Journal of Cancer Supplements*, Vol. 7, No 3, September 2009, Page 6, Abstract 10LBA) A similar benefit to panitumumab was not observed in patients with *KRAS* mutant tumors. These data provide further support to the conclusions made in this manuscript.

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