BRAF Testing in Advanced Colorectal Cancer: Is It Ready for Prime Time?

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Abstract: Given that *KRAS* mutant colorectal tumors do not respond to anti-EGFR monoclonal antibodies such as cetuximab or panitumumab, it is now standard that all patients with metastatic colorectal cancer who are candidates for these therapies undergo KRAS testing. *BRAF* encodes a protein kinase, which is involved in intracellular signaling and cell growth and is a principal downstream effector of KRAS. *BRAF* is now increasingly being investigated in metastatic colorectal carcinoma. *BRAF* mutations occur in less than 10–15% of tumors and appear to be poor prognostic markers. However the predictive nature of this biomarker is yet undefined. This article will review the evidence behind both *KRAS* and *BRAF* testing in metastatic colorectal cancer.

Introduction

The advent of target-specific cancer therapeutics has remarkably improved the outcomes of patients with metastatic colorectal cancer (mCRC). The 3 monoclonal antibodies that are approved in treatment of mCRC include cetuximab (Erbitux, ImClone) and panitumumab (Vectibix, Amgen), which are monoclonal antibodies against epidermal growth factor receptor (EGFR), and bevacizumab (Avastin, Genentech), which is a monoclonal antibody against vascular endothelial growth (VEGF) receptor. From recent trials, the predictive role of Kirsten rat sarcoma viral oncogene homolog (*KRAS*) has been well validated. It has been shown that patients with *KRAS* mutant tumors do not respond to cetuximab and panitumumab and, therefore, it is now recommended that all patients with mCRC who are candidates for anti-EGFR monoclonal antibody therapy have their tumors tested for *KRAS* mutation.[1-3] However, even with *KRAS* mutational testing, there are still many patients with *KRAS* wild-type tumors that do not respond to treatment with cetuximab or panitumumab.[1,3-5] This suggests that other factors such as alterations in other EGFR effectors, including members of the *RAS*-mitogen activated protein kinase (MAPK) or phosphoinositide 3-kinase (P13K) pathways, could drive resistance to anti-EGFR therapy.[6] V-raf murine sarcoma viral oncogene homolog B1 (*BRAF*) is a principle downstream effector of *KRAS*, but the relationship between *BRAF* and *KRAS* has not been completely

elucidated. The National Comprehensive Cancer Network (NCCN) has recently recommended consideration of *BRAF* testing for *KRAS* wild-type tumors, but the clinical utility of this information is still unknown.[7] This article will discuss the data currently available on *BRAF* testing and how they apply to clinical practice.

EGFR

EGFR is a receptor tyrosine kinase and composed of an extracellular ligand binding domain, a lipophilic transmembrane domain, and an intracellular tyrosine kinase domain. EGFR is the link between the extracellular space and the intracellular signal transduction, which regulates nuclear process involved in cell growth, differentiation, survival, cell cycle progression, and angiogenesis.[8] The EGFR signals through the MAPK pathway that regulates the G1 checkpoint and helps control cellular proliferation. MAPK activation is a common property of cancer, and often occurs due to activating mutations in the *RAS* and *BRAF* genes, which are downstream from EGFR.[9]

Immunohistochemistry (IHC) of CRC tumors indicates that EGFR protein expression occurs in 60–80% of CRC.[10] The BOND (Bowel Oncology with Cetuximab Antibody) study, which compared irinotecan plus cetuximab with cetuximab alone in irinotecan-refractory patients, showed response rates of 22.9% and 10.8% in the combination and monotherapy group, respectively.[11] Entry criteria for this study required EGFR expression by IHC in the primary tumor or metastatic lesion. Data from the BOND study, however, showed that the degree of EGFR expression determined by staining intensity or percentage of staining cells did not correlate with response. In addition, other studies have shown a response rate of up to 25% in EGFR-negative CRC patients, indicating that analysis of IHC does not have predictive value.[12-14] Thus, other markers for response to anti-EGFR therapy such as *KRAS* have been evaluated.

KRAS

KRAS protein is a GTPase and is involved in many signal transduction pathways.[15] *KRAS* is part of the downstream signal transduction pathway of EGFR and acts as a molecular on/off switch.[16] Once it is turned on, it recruits and activates proteins necessary for the propagation of growth factor and other receptor signals, such as c-Raf and PI3K. When the EGFR pathway is activated, small G-protein *RAS*, in concert with the protein kinase RAF, activates the MAPK cascade.[17] Mutations of *KRAS* suggest that tumors will not benefit from anti-EGFR agents because the activating mutation occurs downstream from the target of anti-EGFR therapy.[9] The *KRAS* mutation occurs early in oncogenesis and seems to be preserved as the tumor progresses.

KRAS mutations are found in up to 40% of mCRC tumors.[9] Point mutations of the *KRAS* gene have been identified most commonly in codons 12 and 13 and less commonly in codon 61.[18] Randomized controlled trials of cetuximab or panitumumab with or without combination chemotherapy have evaluated outcomes for patients with mCRC harboring *KRAS* mutation (Table 1).[1,5,9,19-22]

The phase III CRYSTAL (Cetuximab Combined with Irinotecan in First Line Therapy for Metastatic Colorectal Cancer) trial evaluated the efficacy of an irinotecan-based regimen with or without cetuximab in first-line, advanced CRC.[3] Overall, the results showed that cetuximab combined with 5-fluorouracil (5-FU), leucovorin, and irinotecan (FOLFIRI) had superior median progression-free survival (PFS) compared to FOLFIRI alone (8.9 vs 8 months; P=.0479). In patients with wild-type *KRAS*, the addition of cetuximab to FOLFIRI significantly improved the median PFS to 9.9 months (P=.017) as well as the objective response rate (ORR) to 59% (P=.0025). However, patients with mutant *KRAS* did not derive any clinical benefit from the addition of cetuximab.

Similarly, a first-line phase II study using an oxaliplatin-based regimen with or without cetuximab (OPUS study) showed that patients with mutant *KRAS* receiving cetuximab have a decreased median PFS compared to the control (5.5 vs 8.6 months; P=.0192), and a trend towards a decreased ORR (32.7% vs 48.9%; P=.106).[4] In a phase III trial by Karapetis and colleagues, patients who were heavily pretreated were randomized to cetuximab alone or best supportive care.[5] In this trial, 394 of 572 patients (68.9%) with CRC had *KRAS* mutational status analyzed. For patients with wild-type *KRAS* tumors, treatment with cetuximab as compared with supportive care alone significantly improved overall survival (OS, 9.5 vs 4.8 months; P<.001) and PFS (median, 3.7 vs 1.9 months; P<.001).

A phase III trial compared panitumumab to best supportive care in patients with mCRC who progressed after standard chemotherapy.[23] In this trial, there was a PFS benefit (8 vs 7.3 weeks; P<.0001) in the panitumumab arm, but no difference in OS, as crossover was allowed in this study. Amado and colleagues also examined the *KRAS* status and the effectiveness of panitumumab.[1] In the group of patients receiving panitumumab, benefit was seen only in patients with wild-type *KRAS*, shown by the increase in PFS (12.3 vs 7.3 weeks; P<.0001) and OS (8.1 vs 7 months).

Another similar study by Hecht and coworkers looked at the interaction of *KRAS* status and the efficacy of panitumumab in chemorefractory mCRC patients with low (1–9%) or negative (<1%) EGFR tumor cell

Table 1. Clinical Trial Evidence of the Response of Anti-EGFR Monoclonal Antibodies as Related to KRAS Mutational Status in Advanced Colorectal Carcinoma

Author/ Study	Treatment	*KRAS* Wild-type				*KRAS* Mutated		
		Variable	Antibody Arm	Control Arm	*P* value/HR	Antibody Arm	Control Arm	*P* value/HR
Van Cutsem et al[3] (CRYSTAL trial)	FOLFIRI ± cetuximab (First-line)	No. of patients	172	176		105	87	
		RR, %	59.3	43.2	*P*=.0025	36.2	40.2	*P*=.46
		Median PFS, mo	9.9	8.7	*P*=.017	7.6	8.1	*P*=.47
Bokemeyer et al[4] (OPUS trial)	FOLFOX ± cetuximab (First-line)	No. of patients	61	73		52	47	
		RR, %	61	37	*P*=.011	33	49	*P*=.106
		Median PFS, mo	7.7	7.2	*P*=.016; HR, 0.057	5.5	8.6	*P*=.0192; HR, 1.83
Karapetis et al[5]	Cetuximab vs supportive care (Chemo-refractory)	No. of patients	117	113		81	83	
		RR, %	13	0		1.2	0	
		Median PFS, mo	3.7	1.9	*P*<.001; HR, 0.4 (95% CI, 0.3–0.54)	1.8	1.8	*P*=.96; HR, 0.99
Amado et al[1]	Panitumumab vs supportive care (Chemo-refractory)	No. of patients	124	119		84	100	
		RR, %	17	0		0	0	
		Median PFS, wk	12.3	7.3	*P*<.0001; HR, 0.45	7.4	7.3	HR, 0.99
Hecht et al[24]	Panitumumab (Chemo-refractory)	No. of patients	94			76		
		Median PFS, mo	15			7.1		
		OS, mo	54			29		
Tol et al[44,45] (CAIRO2 study)	Capecitabine, oxaliplatin, bevacizumab, ± cetuximab	RR, %	61.4	50	*P*=.06	45.9	59.2	*P*=.03
		Median PFS, mo	10.5	10.6	*P*=.3	8.1	12.5	*P*=.003
		Median OS, mo	21.8	22.4	*P*=.64	17.2	24.9	*P*=.03
Douillard et al[25] (PRIME trial)	FOLFOX ± panitumumab (First-line)	No. of patients	331	331		219	221	
		Median PFS, mo	9.6	8	*P*=.0234; HR, 0.80	7.3	8.8	*P*=.0277; HR, 1.29
Peeters et al[26,27] (181 study)	FOLFIRI ± panitumumab (First-line)	No. of patients	303	294		238	248	
		PFS, mo	5.9	3.9	*P*=.004, HR, 0.73			
		OS, mo	14.5	12.5	*P*=.115, HR, 0.85			

FOLFIRI=fluorouracil, leucovorin, irinotecan; FOLFOX=fluorouracil, leucovorin, oxaliplatin; OS=overall survival; PFS=progression-free survival; RR=response rate.

expression by IHC.[24] In this study, a response to panitumumab was observed in both the EGFR negative and EGFR low cohorts. When the *KRAS* analysis was examined, there was a clear advantage in patients with wild-type *KRAS*, as PFS (15 vs 7.1 months) and OS (54.0 vs 29.1 months) were both doubled compared to the *KRAS* mutant population.

The PRIME (Panitumumab Randomized Trial In Combination With Chemotherapy for Metastatic Colorectal Cancer to Determine Efficacy) trial is a

phase III, multicenter, European study in which patients were randomized to 5-FU, leucovorin, and oxaliplatin (FOLFOX4) with or without panitumumab as first-line therapy for mCRC.[25] In patients with wild-type *KRAS* tumors, panitumumab statistically improved PFS when added to FOLFOX4 (median, 9.6 vs 8.0 months; hazard ratio [HR], 0.80; *P*=.02). For the mutant *KRAS* tumors, panitumumab caused a decrease in PFS when added to FOLFOX4, as compared to FOLFOX4 alone (median, 7.3 vs 8.8 months; HR, 1.29; *P*=.02). These results again correlate with the previously reported data.

The 181 study is a randomized, multicenter, phase III study comparing FOLFIRI plus panitumumab to FOLFIRI alone as second-line therapy for mCRC.[26,27] The study was amended to evaluate *KRAS* status. Correlating with previous studies, there was no difference in PFS, OS, or response rate among patients with *KRAS* mutant tumors. *KRAS* wild-type patients had a statistically significant median PFS of 5.9 months and 3.9 months for FOLFIRI plus panitumumab and FOLFIRI alone, respectively (HR 0.73; *P*=.004). The OS for this same group was not statistically significant (14.5 vs 12.5 months; *P*=.12). The improvement in PFS only in the *KRAS* wild-type group is yet more proof that *KRAS* is a predictive marker for response to anti-EGFR therapy.

KRAS testing has proven to be a breakthrough identification biomarker, which has been reproduced and validated by multiple clinical studies. However, *KRAS* is a negative predictive marker. When the previous studies were reviewed, the ORR for *KRAS* wild-type patients was only 17% (vs 0% in *KRAS* mutated patients) for panitumumab monotherapy[1] and 12.8% (vs 1.2% in *KRAS* mutated patients) for cetuximab monotherapy.[5] Thus, other signal transduction pathways, which impact the efficacy of anti-EFGR therapy, such as *BRAF*, *PIK3CA* mutations, c-met, PTEN, hepatocyte growth factor, and insulin growth factor receptor pathways, are being evaluated.[28,29]

The prognostic value of *KRAS* in mCRC is not yet established, as trials have conflicting results. In the phase III trial by Karapetis and colleagues, there was no difference in survival between *KRAS* wild-type and *KRAS* mutant patients who received supportive care.[5] In the phase III trial by Hurwitz and associates evaluating irinotecan, fluorouracil, and leucovorin (IFL) with or without bevacizumab, *KRAS* analysis was performed in 230 patients.[30,31] For patients treated with IFL plus placebo, the median PFS was 7.4 months for *KRAS* wild-type patients and 5.5 months for *KRAS* mutant patients (HR, 0.69; 95% confidence interval [CI], 0.44–1.08; *P*=.11) with OS of 17.6 months and 13.6 months, respectively. Although PFS and OS were longer in the *KRAS* wild-type group, these were not statistically significant. In the updated analysis of the CRYSTAL trial, the *KRAS* wild-type patients did better overall in regard to PFS and OS compared to the *KRAS* mutant patients.[32] The OS in the control group was 20.0 versus 16.7 months for *KRAS* wild-type versus mutant patients, though the study was not statistically designed to show this difference. Thus, the prognostic value of *KRAS* in colon cancer is still uncertain and will need to be evaluated in future and ongoing studies.

BRAF

BRAF encodes a protein kinase, which is involved in intracellular signaling and cell growth. The gene product is also a principal downstream effector of *KRAS* within the *RAS/RAF/MAPK* pathway.[33] Activation of *KRAS* has been studied extensively, but *BRAF* has been only marginally investigated. *BRAF* mutations are seen most commonly in melanoma, but have also been detected in lung, thyroid, acute leukemias, lymphoma, and colon cancer.[34-38] The high frequency of *BRAF* mutations in human cancer suggests that it may function as an oncogene, and plays an important role in both tumor initiation and maintenance of growth.[39]

The *BRAF* gene may be mutated anywhere along its sequence, but the most commonly tested areas include exon 11 codon 468, exon 15 codon 596, and exon 15 codon 600. Tumor DNA may be extracted from fresh, frozen, or paraffin-embedded tissue and amplified by polymerase chain reaction techniques. Sequence analysis of the above hot spots determines the presence of a mutation. More than 95% of *BRAF* mutations in CRC occur at exon 15 as a point mutation, V600E.[21] This mutation results in constitutive activation of the *BRAF* kinase and promotes cell transformation.[33,40]

The incidence of *BRAF* mutations varies by the type of CRC. *BRAF* has been associated with mismatch repair deficient colon cancers, with approximately 40% of microsatellite instability (MSI)-high tumors having a *BRAF* mutation compared to nearly 5% of microsatellite stable tumors.[41] *BRAF* mutations in rectal cancer are extremely rare, as was seen in a study by Kalady and colleagues; they found no mutation in 89 rectal cancer cases compared to a 17% incidence in 268 colon tumors.[42] In fact, mutations in *BRAF* are found in less than 10–15% of mCRC cases.[5,19,43,44] Mutations in *KRAS* and *BRAF* appear to be mutually exclusive. In a study of 113 patients with mCRC, *KRAS* mutation was detected in 30% of the patients. The *BRAF* V600E mutation was detected in 11 of 79 patients who had wild-type *KRAS*.[19]

Presence of *BRAF* mutation status in mCRC has been shown to impact the benefit to anti-EGFR antibodies (Table 2). Di Nicolantonio and coauthors looked retrospectively at 113 patients with mCRC who had received either cetuximab or panitumumab.[19] None of the *BRAF* mutated patients responded to treatment, whereas none

Table 2. Clinical Trial Evidence of the Response of Anti-EGFR Monoclonal Antibodies as Related to BRAF Mutational Status in Advanced Colorectal Carcinoma

Author/Study	Treatment	Variable	BRAF Wild-type	BRAF Mutated	*P* value/HR
Di Nicolantonio et al[19]	Panitumumab or cetuximab	No. of pts	68/79	11/79	
		RR	22/68 (32.4%)	0/11 (0%)	*P*=.029
		PFS	NA	NA	*P*=.001
		OS	NA	NA	*P*<.0001
Loupakis et al[43]	Irinotecan + cetuximab	No. of pts	74/87	13/87	
		RR	24/74 (32%)	0/13 (0%)	*P*=.016
		PFS			*P*=.073/0.59
Tol et al[44,45] CAIRO2 trial	Capecitabine, oxaliplatin, bevacizumab, ± cetuximab	No. of pts (T)	231	28	
		No. of pts (C)	243	17	
		PFS, mo (T)	10.4	6.6	*P*=.010
		PFS, mo (C)	12.2	5.9	*P*=.003
		OS, mo (T)	21.5	15.2	*P*=.001
		OS, mo (C)	24.6	15	*P*=.002
Van Cutsem et al[3,32] CRYSTAL trial	FOLFOX ± cetuximab	No. of pts (KRAS wt only)	566/625	59/625	
		PFS, mo, T/C	10.9/8.8 (*P*=.0016)	8/5.6 (*P*=.86)	
		OS, mo, T/C	25.1/21.6 (*P*=.0549)	14.1/10.3 (*P*=.744)	

C=control arm; FOLFOX=fluorouracil, leucovorin, oxaliplatin; OS=overall survival; PFS=progression-free survival; RR=response rate; T=treatment arm; wt=wild-type.

of the responders carried *BRAF* mutations (*P*=.029). In this study, *BRAF* mutation was a poor prognostic marker, as patients had shorter PFS and OS. Similar to this study, Loupakis and colleagues looked retrospectively at *BRAF* status in patients receiving irinotecan and cetuximab.[43] Among the 87 patients in the study population, *BRAF* was mutated in 13 cases, and none of those patients responded to chemotherapy, compared to a 32% response rate in patients with wild-type *BRAF*. Once again, *BRAF* mutation was associated with a trend towards shorter PFS (HR, 0.59; *P*=.073).

In the CAIRO 2 (Capecitabine, Irinotecan, Oxaliplatin) study, 755 patients with mCRC were randomized to capecitabine, oxaliplatin, and bevacizumab, or the same regimen plus cetuximab.[45] The cetuximab arm in this study had a shorter PFS and inferior quality of life. A retrospective analysis of *BRAF* V600E mutation was performed in 516 available tumors. *BRAF* mutations were found in 45 of these tumors (8.7%).[44] Patients with *BRAF* mutations had a shorter median PFS and OS compared to wild-type *BRAF* tumors in both treatment groups. Also, subset analysis was done to determine outcome depending on *KRAS* status. Patients with wild-type *KRAS* had no difference in PFS or OS. However, patients with *KRAS* mutant tumors who received cetuximab had shorter median PFS (8.1 months) compared to patients who received no cetuximab (12.5 months; *P*=.003). The authors concluded that *BRAF* mutation is a poor prognostic marker regardless of the treatment arm.

In a retrospective study of data from 2 institutions by Souglakos and coworkers, the prognostic and predictive value of *KRAS*, *PIK3CA*, and *BRAF* mutations for clinical outcomes in response to active agents in the treatment of mCRC was evaluated.[28] Mutational status was determined in 168 patients; *KRAS*, *BRAF,* and *PIK3CA* mutations were present in 62 (37%), 13 (8%), and 26 (15%) patients, respectively. Multivariate analysis discovered *BRAF* mutation as an independent prognostic factor for decreased survival (HR 4.0; 95% CI, 2.1–7.6) and a lower PFS (HR, 4.0; 95% CI, 2.2–7.4). In this study, 92 patients were treated using chemotherapy and cetuximab. None of the 9 patients with *BRAF* mutations

responded to cetuximab, whereas 14 of the 83 *BRAF* wild-type patients responded. For those who received chemotherapy with or without bevacizumab, patients with *BRAF* mutations did poorly regardless of the type of chemotherapy they received. Overall, this study showed that *BRAF* mutations carry an especially poor prognosis regardless of treatment choice.

In a recent retrospective article by Sartore-Bianchi and associates, a comprehensive analysis of *KRAS*, *BRAF*, *PIK3CA* mutations, and PTEN expression in mCRC patients treated with cetuximab or panitumumab was conducted.[14] Of all patients, 96 had wild-type *KRAS* and underwent testing for *BRAF* and *PIK3CA* mutations and PTEN expression. A multivariate analysis found that the loss of PTEN confirmed a significant association with lack of response (P<.001), whereas *BRAF* (P=.265) and *PIK3CA* (P=.075) were not significant. Survival analyses demonstrated that *BRAF* mutations (HR, 3.75; P=.015) and loss of PTEN (HR, 0.43; P=.009), but not *PIK3CA* mutations (HR, 1.20; P=.672), were significantly associated with decreased OS, whereas none of these alterations was significantly associated with PFS.

The previously described CRYSTAL trial randomized 1,198 patients with untreated mCRC to FOLFIRI with or without cetuximab.[3] The benefit of cetuximab was limited to the wild-type *KRAS* patients. Recent analysis of the CRYSTAL trial, reported at the American Society of Clinical Oncology (ASCO) 2010 Gastrointestinal Cancers Symposium, evaluated the influence of *KRAS* and *BRAF* biomarkers on outcome.[32] As expected, there was a statistically significant improvement in response rate and PFS for the *KRAS* wild-type/*BRAF* wild-type patients receiving cetuximab. As shown by several of the above reviewed trials, *BRAF*-mutant patients overall have a poor prognosis. Prior to this analysis, it was also believed that *BRAF*-mutant patients would be unlikely to respond to anti-EGFR therapy. Of the *KRAS* wild-type and *BRAF* mutant patients in the CRYSTAL trial, the OS for FOLFIRI plus cetuximab and FOLFIRI alone was 14.1 and 10.3 months, respectively (P=.7440). Although this was not statistically significant, there was an overall trend towards improved OS, PFS, and response, suggesting that *KRAS* wild-type/*BRAF* mutant patients may benefit from treatment with anti-EGFR therapy.

Also at the recent ASCO Gastrointestinal Cancers Symposium, a meta-analysis of the CRYSTAL and OPUS trials evaluated OS, PFS, and overall response with respect to *KRAS* and *BRAF* tumor mutation status.[46] The results showed that the addition of cetuximab to chemotherapy for *KRAS* wild-type tumors (845 patients) produced a reduced risk of disease progression and increased overall response and OS (HR, 0.81; 95% CI, 0.69–0.9; P<.0001) compared to chemotherapy alone, which coincides with results from previous studies. The final analysis of *BRAF* mutational status is still pending.

The role of *BRAF* as a prognostic marker for early stage colon cancer is less studied. PETAACC-3 (The Pan-European Trials in Adjuvant Colon Cancer) was a large, randomized phase III trial, which assessed the role of irinotecan added to fluorouracil/leucovorin as adjuvant treatment for stage II and III colon cancer.[47] The resection specimens of 1,564 patients were prospectively collected. These were analyzed for *KRAS* and *BRAF* mutations.[48] *BRAF* mutations were significantly associated with right-sided tumors, older age, high grade, and MSI-high tumors. *BRAF* was a prognostic marker for OS in MSI-low and MSI-stable tumors, though *KRAS* was not. Another study, which retrospectively tested 649 colon cancers (stage I–IV), evaluated *BRAF*, *KRAS*, MSI, and CpG island methylator phenotype (CIMP).[49] Colon cancers that exhibit widespread promoter methylation, also referred to as *CIMP*, have been associated with MSI and *BRAF* mutations.[50-52] As previously seen, *BRAF* mutation in this study was associated with high mortality. CIMP-high was an independent predictor of low colon cancer–specific mortality. For patients who had a *BRAF* mutation and CIMP-high, the adverse influence of *BRAF* seemed to be overridden by the good prognosis of CIMP-high.

A potential problem faced by oncologists is turnaround time, as it may take weeks to obtain final test results or *KRAS* and *BRAF* tests. Currently, there are commercial kits in development that will test for *KRAS* and automatically test for *BRAF* if patients harbor wild-type *KRAS*.

Conclusion

KRAS testing highlights the importance of further development of diagnostic markers to predict response to targeted therapy. *KRAS* testing has been an important step forward in the management of mCRC, and it is clear that only patients with *KRAS* wild-type tumors should be considered for anti-EGFR therapy. Currently, commercial tests for *BRAF* are available; however, there is no standardized kit approved by the US Food and Drug Administration to test for *BRAF* mutation. NCCN guidelines recommend that patients with metastatic colorectal disease have *BRAF* gene status determined as part of their workup when the *KRAS* gene is not mutated.[7] The guidelines also state that patients with a known V600E *BRAF* mutation should not be treated with anti-EGFR monoclonal antibodies.

The available data for *BRAF* mutations predicting response to anti-EGFR therapy are limited by retrospective analysis and small numbers of patients with *BRAF* mutations. However, it seems clear that this mutation is

a poor prognostic marker, as it is associated with shorter PFS and OS regardless of treatment. Given the recent results of the retrospective analysis of the CRYSTAL trial, it cannot be assumed that *BRAF* mutational status is predictive for response to anti-EGFR therapy. On the contrary, the CRYSTAL data suggest that *KRAS* wild-type/*BRAF* mutant patients may actually respond to anti-EGFR therapy.

Thus, based on the available data, *BRAF* testing should not be routinely performed outside of a clinical trial. With the evidence we currently have, it is unclear how we can apply the results of *BRAF* testing to the treatment of advanced CRC. Although patients with *BRAF* mutations have a poor prognosis, more information is necessary to determine the ability of *BRAF* testing to predict response to anti-EGFR therapy. It seems impractical to suggest that we need prospective studies evaluating *BRAF* mutational testing with anti-EGFR therapies prior to recommending its use in clinical practice, given that it occurs in less than 10–15% of patients. It may be reasonable to proceed with a meta-analysis of the current anti-EGFR therapy trials to evaluate *BRAF* mutations.

In conclusion, *BRAF* mutation is a negative prognostic marker in patients with mCRC and is associated with a shorter PFS and OS. In the future, comprehensive dissection of the EGFR signaling pathways may be needed to select mCRC patients who will respond to cetuximab- or panitumumab-based therapies.

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