

RAS and BRAF in metastatic colorectal cancer management

Jun Gong¹, May Cho¹, Marwan Fakih²

¹Department of Medical Oncology, City of Hope National Medical Center, Duarte, CA, USA; ²Medical Oncology and Experimental Therapeutics, City of Hope Comprehensive Cancer Center, Duarte, CA, USA

Contributions: (I) Conception and design: All authors; (II) Administrative support: None; (III) Provision of study materials or patients: None; (IV) Collection and assembly of data: None; (V) Data analysis and interpretation: None; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

Correspondence to: Marwan Fakih, Medical Oncology and Experimental Therapeutics, City of Hope Comprehensive Cancer Center, Building 51, Room 127, 1500 E Duarte St, Duarte, CA 91010, USA. Email: mfakih@coh.org.

Abstract: The treatment of metastatic colorectal cancer (mCRC) has been further refined with the development of monoclonal antibodies, cetuximab and panitumumab, towards the epidermal growth factor receptor (EGFR). Anti-EGFR therapy has afforded improved survival in those with wild-type RAS mCRC but provides no benefit and even harm in those with RAS-mutant tumors. *BRAF* mutations have also been shown to predict lack of clinically meaningful benefit to anti-EGFR therapy in mCRC. Mechanisms of resistance to EGFR blockade in wild-type *RAS* or *BRAF* metastatic colorectal tumors appear to converge on the mitogen-activated protein kinase (MAPK) signaling pathway. Clinical trials involving combined BRAF, EGFR, and/or MAPK kinase (MEK) inhibition have shown promising activity in *BRAF*-mutant mCRC. Here, we review pivotal clinical trials that have redefined our treatment approach in mCRC with respect to anti-EGFR therapy based on *RAS* and *BRAF* mutation status. Future studies will likely focus on improving efficacy of anti-EGFR-based therapy in mCRC through sustained MAPK pathway inhibition.

Keywords: RAS; BRAF; cetuximab; panitumumab; colorectal cancer (CRC)

Submitted Mar 30, 2016. Accepted for publication May 17, 2016.

doi: 10.21037/jgo.2016.06.12

View this article at: <http://dx.doi.org/10.21037/jgo.2016.06.12>

Introduction

Colorectal cancer (CRC) is the third leading cause of cancer death in both men and women in the United States with an estimated 134,490 new cases and 49,190 deaths in 2016 (1).

Approximately 25% of patients present with metastatic disease at diagnosis, and about 50% of patients with CRC will eventually develop metastases (2). Since the 1950s and until the last two decades, 5-fluorouracil (5-FU) with leucovorin (LV) remained the standard therapy in advanced CRC (3,4). The current approach to treating metastatic CRC (mCRC) favors the use of combination cytotoxic therapy including 5-FU, LV, and irinotecan (FOLFIRI), 5-FU, LV, and oxaliplatin (FOLFOX), capecitabine and oxaliplatin (XELOX), or 5-FU, LV, oxaliplatin, and irinotecan (FOLFOXIRI) in the first-line setting (5-9). The addition of targeted agents against

angiogenesis, in particular the vascular endothelial growth factor A (VEGF-A) inhibitor bevacizumab, to combination chemotherapy has afforded improved outcomes in mCRC in the first-line and second-line settings (10-12).

The treatment landscape in mCRC has been further refined with the advent of targeted therapy against the epidermal growth factor receptor (EGFR). Activation of the mitogen-activated protein kinase (MAPK) signaling pathway has long been recognized to promote colorectal carcinogenesis by affecting cell growth, proliferation, and survival (13). Activation of receptor tyrosine kinases including EGFR and related downstream signaling through RAS, RAF, MAPK kinase (MEK), and extracellular-signal-regulated kinase (ERK) comprise key steps that can be involved along the MAPK tumorigenic cascade. Notably, the presence of activating mutations in *RAS* and *BRAF* are associated with poorer prognosis and have been identified

as predictors of resistance to anti-EGFR monoclonal antibodies, cetuximab and panitumumab, in mCRC (14–16). Findings from large, randomized clinical trials have recently confirmed the survival benefits afforded by the addition of anti-EGFR monoclonal antibodies to standard combination chemotherapy in *RAS* and *BRAF* wild-type metastatic colorectal tumors. Here, we review data from pivotal clinical trials that have redefined our treatment approach in mCRC with respect to *RAS* and *BRAF* mutation status.

RAS mutation status as a biomarker of response to anti-EGFR therapy

Oncogenic *RAS* mutations have historically been present in approximately 40–50% of CRC cases (17). In a recent pooled analysis, the prevalence of *RAS* mutations in mCRC has been shown to be as high as 55.9% with mutations in *KRAS* exon 2 being the most common (42.6%) followed by *KRAS* exon 3 (3.8%), *KRAS* exon 4 (6.2%), *NRAS* exon 2 (2.9%), *NRAS* exon3 (4.2%), and *NRAS* exon4 (0.3%) mutations (18). Mutations in codons G12D, G12V, and G12C were most common for *KRAS* exon 2, codons Q61H and Q61R for *KRAS* exon 3, codons A146T and A146V for *KRAS* exon 4, codon G12D for *NRAS* exon2, codons Q61K and Q61R for *NRAS* exon3, and codon A146T for *NRAS* exon4. In the initial RASCAL study, the presence of a *KRAS* mutation was associated with poorer overall survival (OS) and increased risk of relapse in mCRC (19). In addition, an analysis of the N0147 trial has shown an increased relapse rate for *RAS*-mutant CRC [codon 12 hazard ratio (HR) 1.52; 95% confidence interval (CI), 1.28–1.80; $P < 0.0001$ and codon 13 HR 1.36; 95% CI, 1.04–1.77; $P = 0.0248$] in comparison to *RAS*-wild-type patients (20). The prognostic value of *KRAS* mutation in the metastatic disease setting is more controversial, as many non-EGFR containing arms of treatment have failed to show a difference in outcome between *KRAS*-mutant and *KRAS*-wild-type CRC (21–23). Since then, seminal clinical trials have shown that *RAS* mutation status also predicts response to anti-EGFR therapy, in particular cetuximab and panitumumab, in first-line and beyond settings in the treatment of mCRC.

Chemotherapy refractory settings

Cetuximab first gained Food and Drug Administration (FDA) approval on the basis of the BOND trial. This multicenter, randomized control trial (RCT) investigated cetuximab given at initial dose of 400 mg/m² followed by

weekly infusions of 250 mg/m² alone or in combination with irinotecan in 329 patients with EGFR-expressing mCRC who progressed on one or more lines of irinotecan-based chemotherapy (24). Cetuximab + irinotecan demonstrated a significantly improved overall response rate (ORR) and median progression-free survival (PFS) compared to cetuximab alone (Table 1). Grade 3 or 4 adverse events (AEs) were more frequent in the combination therapy arm including diarrhea (21.2%) and neutropenia (9.4%) than the monotherapy arm, but their incidence and severity were similar to those expected with irinotecan alone. The phase III CO.17 study randomized 572 patients with EGFR-expressing mCRC refractory to chemotherapy to single-agent cetuximab + best supportive care (BSC) *vs.* BSC alone (25). In contrast to the BOND trial, treatment with cetuximab demonstrated significantly improved OS (HR 0.77; 95% CI, 0.64–0.92; $P = 0.005$) as well as PFS and ORR when compared to BSC alone (Table 1). The incidence of grade ≥ 3 AEs were higher with cetuximab treatment with respect to rash (11.8% *vs.* 0.4%), infection without neutropenia (12.8% *vs.* 5.5%), confusion (5.6% *vs.* 2.2%), pain (14.9% *vs.* 7.3%), and hypomagnesemia (5.8% *vs.* 0.0%). The presence of a grade ≥ 2 rash was associated with improved survival in the cetuximab arm (HR 0.33; 95% CI, 0.22–0.50; $P < 0.001$).

Notably, these early studies evaluated the efficacy of anti-EGFR therapy in mCRC so long as tumors were EGFR-expressing; the effect of *RAS* mutation status and response to anti-EGFR therapy was not investigated. However, a post hoc analysis of the CO.17 trial involving *KRAS* mutation analysis in 394 tumor specimens collected at the time of diagnosis demonstrated median OS of 4.5 (cetuximab) *vs.* 4.6 months (BSC, HR 0.98; 95% CI, 0.70–1.37; $P = 0.89$) among *KRAS*-mutant tumors and median OS of 9.5 (cetuximab) *vs.* 4.8 months (BSC, HR 0.55; 95% CI, 0.41–0.74; $P < 0.001$) among *KRAS*-wild-type tumors (26). A similar benefit was seen in median PFS between cetuximab (3.7 months) *vs.* BSC (1.9 months; HR 0.40; 95% CI, 0.30–0.54; $P < 0.001$) among *KRAS*-wild-type tumors whereas no benefit in PFS was observed between arms among *KRAS*-mutant tumors. Of note, *KRAS* mutation analysis was limited to codons 12 and 13 of exon 2.

KRAS mutation status has similarly been shown to predict benefit to the anti-EGFR monoclonal antibody, panitumumab, in chemotherapy-resistant mCRC. The phase III 408 study assigned 463 patients with EGFR-expressing mCRC who progressed on ≥ 2 lines of prior chemotherapy to panitumumab [60-minute intravenous

Table 1 KRAS status and anti-EGFR therapy in metastatic colorectal cancer in chemotherapy refractory settings

Study	n= size	Arms	ORR	PFS	OS
BOND (24)	329	Cetuximab + irinotecan vs. cetuximab	22.9% (95% CI, 17.5–29.1) vs. 10.8% (95% CI, 5.7–18.1, P=0.007)	Median 4.1 vs. 1.5 months (P<0.001)	Median 8.6 vs. 6.9 months (P=0.48)
CO.17 (25)	572	Cetuximab + BSC vs. BSC	PR 8.0% vs. 0.0% (P<0.001); SD 31.4% vs. 10.9% (P<0.001)	HR 0.68 (95% CI, 0.57–0.80; P<0.001)	HR 0.77 (95% CI, 0.64–0.92; P=0.005)
CO.17 post hoc analysis (26)	394	Cetuximab + BSC vs. BSC	KRAS-wt: 12.8% vs. 0.0% (P value NR)	KRAS-wt: median 3.7 vs. 1.9 months (HR 0.40; 95% CI, 0.30–0.54; P<0.001)	KRAS-wt: median 9.5 vs. 4.8 months (HR 0.55; 95% CI, 0.41–0.74; P<0.001)
408 (27)	463	Panitumumab + BSC vs. BSC	10% vs. 0.0% (P<0.0001)	HR 0.54 (95% CI, 0.44–0.66; P<0.0001)	HR 1.00 (95% CI, 0.82–1.22; P=0.81)
408 post hoc analysis (28)	427	Panitumumab + BSC vs. BSC	17% (KRAS-wt) vs. 0% (KRAS-mutant, P value NR, both arms combined)	KRAS-wt: median 12.3 vs. 7.3 weeks (HR 0.45; 95% CI, 0.34–0.59); KRAS-mutant: median 7.4 vs. 7.3 weeks (HR 0.99; 95% CI, 0.73–1.36; P<0.0001)	KRAS-wt vs. KRAS-mutant: HR, 0.67 (95% CI, 0.55–0.82; P value NR, both arms combined)
20100007 (29)	377	Panitumumab + BSC vs. BSC	KRAS-wt (exon 2): 27.0% vs. 1.6% (HR 24.9; 95% CI, 7.5–123.8; P<0.0001); RAS-wt (exons 3,4 KRAS and NRAS): 31.0% vs. 2.3 (HR 20.0; 95% CI, 5.9–101.6; P<0.0001)	KRAS-wt (exon 2): 3.6 vs. 1.7 months (HR 0.51; 95% CI, 0.41–0.64; P<0.0001); RAS-wt (exons 3,4 KRAS and NRAS): 5.2 vs. 1.7 months (HR 0.46; 95% CI, 0.35–0.59; P<0.0001)	KRAS-wt (exon 2): 10.0 vs. 7.4 months (HR 0.73; 95% CI, 0.57–0.93; P=0.0096); RAS-wt (exons 3,4 KRAS and NRAS): 10.0 vs. 6.9 months (HR 0.70; 95% CI, 0.53–0.93; P=0.0135)
ASPECCT (30)	999	Panitumumab vs. cetuximab	OR 1.15 (95% CI, 0.83–1.58; P value NR)	HR 1.00 (95% CI, 0.88–1.14; P value NR)	HR 0.97 (95% CI, 0.84–1.11; P value NR)

ORR, overall response rate; PFS, progression-free survival; OS, overall survival; CI, confidence interval; BSC, best supportive care; PR, partial response; SD, stable disease; HR, hazard ratio; wt, wild-type; NR, not reported; OR, odds ratio.

(IV) infusion at 6 mg/kg once every 2 weeks] + BSC *vs.* BSC alone (27). Treatment with panitumumab conferred an advantage in PFS (HR 0.54; 95% CI, 0.44–0.66; P<0.0001) and ORR (10% *vs.* 0.0%, P<0.0001) over BSC alone. No significant difference in OS (HR 1.00; 95% CI, 0.82–1.22; P=0.81) was observed between arms though 76% of BSC patients were allowed to cross over to the panitumumab arm. Skin-related toxicities were more frequent in the panitumumab arm (90%) than the BSC group (9%). The presence of a grade ≥ 2 rash was associated with improved PFS (HR 0.62; 95% CI, 0.44–0.88) when compared to those treated with panitumumab having grade 1 skin toxicity. A subsequent exploratory analysis of this cohort involving *KRAS* mutation testing (codons 12 and 13) in 427 available tumors showed improved PFS in *KRAS*-wild-type tumors treated with panitumumab (median PFS 12.3 weeks) *vs.* BSC (median PFS 7.3 weeks; HR 0.45; 95% CI, 0.34–0.59) whereas no benefit was seen in the panitumumab arm (median PFS 7.4 weeks) *vs.* BSC

(median PFS 7.3 weeks; HR 0.99; 95% CI, 0.73–1.36) among *KRAS*-mutant tumors (28). This treatment effect on PFS was statistically different between *KRAS*-wild-type tumors *vs.* *KRAS*-mutant tumors (P<0.0001). Response to panitumumab was higher in *KRAS*-wild-type tumors (17%) *vs.* *KRAS*-mutant tumors (0%), and patients with wild-type tumors experienced longer OS compared to mutant tumors after adjustment (HR 0.67; 95% CI, 0.55–0.82, both arms combined). Median OS was improved in those allowed to cross over to the panitumumab arm on progression for wild-type tumors (6.8 months) *vs.* mutant tumors (4.5 months; HR 0.65; 95% CI, 0.47–0.90).

Preliminary results from a recent phase III trial (20100007) involving panitumumab + BSC *vs.* BSC alone in 377 patients with chemorefractory *KRAS*-wild-type (exon 2) mCRC have confirmed advantages across all efficacy endpoints with panitumumab therapy over BSC (Table 1). Notably, cross over to panitumumab was not permitted and

270 patients with *RAS*-wild-type tumors (including exons 3 and 4 of *KRAS* and *NRAS*) were enrolled and similarly experienced benefits across all efficacy endpoints, including OS, when treated with panitumumab over BSC alone (29). The recent phase III ASPECCT trial pitted cetuximab *vs.* panitumumab in a head-to-head investigation in 999 patients with *KRAS*-wild-type (exon 2) mCRC refractory to prior chemotherapy (30). Panitumumab demonstrated non-inferiority to cetuximab in OS (HR 0.97; 95% CI, 0.84–1.11), PFS (HR 1.00; 95% CI, 0.88–1.14), and response rate [odds ratio (OR) 1.15; 95% CI, 0.83–1.58] in this population. The incidence of grade ≥ 3 AEs were similar across treatment arms though grade ≥ 3 skin toxicity was higher with panitumumab (13%) than cetuximab (10%) as well as grade ≥ 3 hypomagnesemia (7% panitumumab *vs.* 3% cetuximab). Grade ≥ 3 infusion reactions occurred less frequently with panitumumab (<0.5%) than cetuximab (2%). Final results have confirmed the non-inferiority of panitumumab to cetuximab for OS in chemotherapy-resistant wild-type *KRAS* (exon 2) mCRC (31).

In chemotherapy refractory settings, cetuximab or panitumumab offers survival advantages in mCRC that are dependent on *KRAS* mutation status. The addition of cetuximab to irinotecan can overcome irinotecan resistance in mCRC previously treated with irinotecan-based chemotherapy. Panitumumab is non-inferior in survival to cetuximab in chemotherapy-resistant wild-type *KRAS* mCRC. The choice of anti-EGFR agent should take into consideration patient factors (e.g., history of infusion reaction) and toxicity profiles of either drug.

First-line settings

The COIN trial randomized 1,630 patients with chemotherapy-naïve mCRC to a control arm [choice of capecitabine 850 mg/m² orally twice daily for 2 weeks + oxaliplatin 130 mg/m² 2-hour infusion (XELOX) every 3 weeks or 5-FU 400 mg/m² bolus followed by 5-FU 2,400 mg/m² infusion over 46 hours + LV 175 mg 2-hour infusion + oxaliplatin 85 mg/m² 2-hour infusion (FOLFOX) every 2 weeks] *vs.* treatment arm (same combination + cetuximab). Tumor samples from 1,316 patients were available for *KRAS* (codons 12, 13, and 61), *NRAS* (codons 12 and 61), and *BRAF* (codons 594 and 600) mutation analysis (32). Among wild-type *KRAS* tumors, the addition of cetuximab to oxaliplatin-based chemotherapy did not significantly improve OS and PFS compared to oxaliplatin-based chemotherapy alone (Table 2). On subgroup analysis,

the addition of cetuximab to FOLFOX demonstrated improved PFS (HR 0.72, 95% CI, 0.53–0.98, $P=0.037$) but not in combination with XELOX. Notably, significantly more grade ≥ 3 toxicities involving skin rash, diarrhea, and hand-foot syndrome were observed with the addition of cetuximab to XELOX, which likely contributed to the lack of benefit in this cohort. OS was 8.8 months for *BRAF* mutants, 13.8 months for *NRAS* mutants, 14.4 months for *KRAS* mutants, and 20.1 months for all wild-type tumors.

Further uncertainty on the benefits of adding cetuximab to oxaliplatin-based chemotherapy in previously untreated mCRC arose from the NORDIC-VII study (33). This phase III trial randomized 571 patients to oxaliplatin 85 mg/m² 30–90 minute infusion on day 1 + 5-FU 500 mg/m² bolus followed by LV 60 mg/m² bolus on days 1–2 (FLOX) every 2 weeks *vs.* cetuximab + FLOX *vs.* cetuximab + intermittent FLOX (discontinued after 16 weeks of treatment). *KRAS* (codons 12 and 13) and *BRAF* (codon 600) mutation analysis was performed on 498 and 457 metastatic colorectal tumors, respectively. Response rates, PFS, and OS did not differ between treatment arms among patients with *KRAS*-wild-type and *KRAS*-mutant tumors (Table 2). Notably, grade ≥ 3 neutropenia was seen in 46–49% of patients across all three treatment arms, and as expected, grade ≥ 3 skin rash was more frequently seen in cetuximab + FLOX (22%) and cetuximab + intermittent FLOX (29%) arms *vs.* FLOX alone (1%, $P<0.01$).

The OPUS study randomized 344 patients with mCRC to first-line oxaliplatin 85 mg/m² 2-hour infusion on day 1 + LV 200 mg/m² 2-hour infusion + 5-FU 400 mg/m² bolus followed by 5-FU 600 mg/m² 22-hour infusion on days 1–2 (FOLFOX4) every 2 weeks *vs.* FOLFOX4 + cetuximab (34). In contrast to the NORDIC-VII study, this open-label, multicenter phase II trial demonstrated a significantly improved ORR and PFS with the addition of cetuximab to FOLFOX4 among wild-type *KRAS* tumors but a worse ORR and PFS with FOLFOX4 + cetuximab, when compared to FOLFOX4 alone, among *KRAS*-mutant tumors (Table 2). The most frequent grade ≥ 3 AEs were neutropenia (30%), rash (11%), and diarrhea (8%) in the cetuximab + FOLFOX4 arm and neutropenia (34%), rash (0.6%), and diarrhea (7%) in the FOLFOX4 alone arm.

The PRIME study provided convincing evidence for improved PFS and OS with the addition of anti-EGFR therapy to chemotherapy in previously untreated patients with wild-type *KRAS* (exon 2) metastatic colorectal tumors (35). In this phase III study, panitumumab was combined with FOLFOX4 and compared to FOLFOX4 alone in 1,183

Table 2 KRAS status and anti-EGFR therapy in metastatic colorectal cancer in first-line settings

Study	n= size	Arms	ORR	PFS	OS
COIN (32)	1,630	XELOX or FOLFOX vs. same combination + cetuximab	57% vs. 64% (P=0.049)	Median 8.6 vs. 8.6 months (HR 0.96; 95% CI, 0.82–1.12; P=0.60); *FOLFOX + cetuximab (HR 0.72; 95% CI, 0.53–0.98; P=0.037)	Median 17.9 vs. 17.0 months (HR 1.04; 95% CI, 0.87–1.23; P=0.67)
NORDIC-VII (33)	571	FLOX vs. cetuximab + FLOX vs. cetuximab + intermittent FLOX	ITT: 41% vs. 49% vs. 47% (OR 1.35; 95% CI, 0.90–2.02; P=0.15); KRAS-wt: 47% vs. 46% vs. 51% (OR 0.96; 95% CI, 0.55–1.69; P=0.89)	ITT: median 7.9 vs. 8.3 vs. 7.3 months (HR 0.89; 95% CI, 0.72–1.11; P=0.31); KRAS-wt: 8.7 vs. 7.9 vs. 7.5 months (HR 1.07; 95% CI, 0.79–1.45; P=0.66)	ITT: median 20.4 vs. 19.7 vs. 20.3 months (HR 1.06; 95% CI, 0.83–1.35; P=0.67); KRAS-wt: 22.0 vs. 20.1 vs. 21.4 months (HR 1.14; 95% CI, 0.80–1.61; P=0.48)
OPUS (34)	344	FOLFOX4 + cetuximab vs. FOLFOX4	KRAS-wt: 61% vs. 37% (OR 2.54; 95% CI, 1.24–5.23; P=0.011); KRAS-mutant: 33% vs. 49% (OR 0.51; 95% CI, 0.22–1.15; P=0.106)	KRAS-wt: median 7.7 vs. 7.2 months (HR 0.57; 95% CI, 0.358–0.907; P=0.0163); KRAS-mutant: 5.5 vs. 8.6 months (HR 1.83; 95% CI, 1.095–3.056; P=0.0192)	NR
PRIME (35)	1,183	Panitumumab + FOLFOX4 vs. FOLFOX4	KRAS-wt: 55% vs. 48% (OR 1.35, P=0.068) KRAS-mutant: 40% vs. 40% (value NR)	KRAS-wt: median 9.6 vs. 8.0 months (HR 0.80; 95% CI, 0.66–0.97; P=0.02); KRAS-mutant: median 7.3 vs. 8.8 months (HR 1.29; 95% CI, 1.04–1.62; P=0.02)	KRAS-wt: median 23.9 vs. 19.7 months (HR 0.83; 95% CI, 0.67–1.02; P=0.072); KRAS-mutant: 15.5 vs. 19.3 months (HR 1.24; 95% CI, 0.98–1.57; P=0.068)
CRYSTAL (36)	1,198	FOLFIRI + cetuximab vs. FOLFOX	KRAS-wt: 57.3% vs. 39.7% (OR 2.069; 95% CI, 1.515–2.826; P<0.001); KRAS-mutant: 31.3% vs. 36.1% (OR 0.822; 95% CI, 0.544–1.242; P=0.35)	KRAS-wt: median 9.9 vs. 8.4 months (HR 0.696; 95% CI, 0.558–0.867; P=0.0012); KRAS-mutant: 7.4 vs. 7.7 months (HR 1.171; 95% CI, 0.887–1.544; P=0.26)	KRAS-wt: median 23.5 vs. 20.0 months (HR 0.796; 95% CI, 0.670–0.946; P=0.0093); KRAS-mutant: 16.2 vs. 16.7 months (HR 1.035; 95% CI, 0.834–1.284; P=0.75)

*, subgroup analysis. ORR, overall response rate; PFS, progression-free survival; OS, overall survival; XELOX, capecitabine + oxaliplatin; FOLFOX, 5-fluorouracil + leucovorin + oxaliplatin; HR, hazard ratio; CI, confidence interval; FLOX, bolus 5-fluorouracil + leucovorin + oxaliplatin; ITT, intention-to-treat; OR, odds ratio; wt, wild-type; NR, not reported; FOLFIRI, 5-fluorouracil + leucovorin + irinotecan.

patients in the first-line setting. Notably, a detriment to PFS and OS was observed with the addition of panitumumab to FOLFOX4 when compared to FOLFOX4 alone among those with *KRAS*-mutant tumors (Table 2). Toxicities were generally comparable across treatment arms except for those known to be associated with anti-EGFR therapy. A later analysis from the PRIME study cohort of 620 tumors absent for *KRAS* mutations in exon 2 identified the presence of extended *RAS* mutations [*KRAS* exon 3 (codon 61) and exon 4 (codons 117 and 146) and *NRAS* exon 2 (codons 12 and 13), exon 3 (codon 61), and exon 4 (codons 117 and 146)] in 17% of patients (37). The presence of extended *RAS* mutations was also associated with a worse outcome in PFS and OS when treated with panitumumab + FOLFOX4, while significant improvement in PFS and OS was seen with

panitumumab + FOLFOX4, when compared to FOLFOX4 alone, in those without *RAS* mutations. Furthermore, a significant improvement in OS was seen with panitumumab + FOLFOX4 (28.3 months) over FOLFOX4 alone (20.9 months) in the wild-type *RAS* and *BRAF* group.

The phase III CRYSTAL study investigated the efficacy of first-line irinotecan 180 mg/m² 30–90 minute infusion on day 1 + LV 200 mg/m² L-form or 400 mg/m² racemic 2-hour infusion followed by 5-FU 400 mg/m² bolus on day 1 followed by 5-FU 2,400 mg/m² 46-hour infusion (FOLFIRI) on days 1–2 every 2 weeks vs. FOLFIRI + cetuximab in 1,198 patients with mCRC (36). Among wild-type *KRAS* tumors (limited to codons 12 and 13), the addition of cetuximab to FOLFIRI conferred advantages to ORR, PFS, and OS when compared to FOLFIRI alone (Table 2). These benefits were

Table 3 KRAS status and anti-EGFR therapy in metastatic colorectal cancer in second-line settings

Study	n= size	Arms	ORR	PFS	OS
EPIC (39)	1,298	Cetuximab + irinotecan vs. irinotecan	16.4% vs. 4.2% (P<0.0001)	Median 4.0 vs. 2.6 months (HR 0.692; 95% CI, 0.617–0.776; P ≤0.0001)	Median 10.7 vs. 10.0 months (HR 0.975; 95% CI, 0.854–1.114; P=0.71)
20050181 (40)	1,186	Panitumumab + FOLFIRI vs. FOLFIRI	KRAS-wt: 35% vs. 10% (P<0.0001); KRAS-mutant: 13% vs. 14% (P=1.0)	KRAS-wt: median 5.9 vs. 3.9 months (HR 0.73; 95% CI, 0.59–0.90; P=0.004); KRAS-mutant: 5.0 vs. 4.9 months (HR 0.85; 95% CI, 0.68–1.06; P=0.14)	KRAS-wt: median 14.5 vs. 12.5 months (HR 0.85; 95% CI, 0.70–1.04; P=0.12); KRAS-mutant: 11.8 vs. 11.1 months (HR 0.94; 95% CI, 0.76–1.15; P=NR)
PICCOLO (41)	460	Panitumumab + irinotecan vs. irinotecan	KRAS-wt: 34% vs. 12% (P<0.0001)	KRAS-wt: HR 0.78; 95% CI, 0.64–0.95; P=0.015	KRAS-wt: median 10.4 vs. 10.9 months (HR 1.01; 95% CI, 0.83–1.23; P=0.91)

ORR, overall response rate; PFS, progression-free survival; OS, overall survival; HR, hazard ratio; CI, confidence interval; FOLFIRI, 5-fluorouracil + leucovorin + irinotecan; wt, wild-type; NR, not reported.

not observed with the addition of cetuximab to FOLFIRI in *KRAS*-mutant tumors. Furthermore, early development of an acne-like rash in patients with wild-type *KRAS* tumors treated with FOLFIRI + cetuximab was associated with improved median OS (26.4 months) *vs.* those without an early rash (19.1 months). The presence of *KRAS* mutations predicted worse OS in both treatment groups compared to *KRAS*-wild-type tumors. The most common grade ≥3 AEs included neutropenia (28.2% FOLFIRI + cetuximab *vs.* 24.9% FOLFIRI), diarrhea (15.7% *vs.* 10.5%), and acne-form rash (16.2% *vs.* 0%). A subsequent post hoc analysis of 430 tumors previously typed as *KRAS* exon 2 wild-type focused on reevaluating for other *RAS* mutations [*KRAS* exons 3 (codons 59 and 61) and 4 (codons 117 and 146) and *NRAS* exons 2 (codons 12 and 13), 3 (codons 59 and 61), and 4 (codons 117 and 146)] from the CRYSTAL study cohort (38). Other *RAS* mutations were identified in 14.7% of this cohort (*KRAS* exon 4 being most common site) and were similarly associated with a lack of benefit across all efficacy endpoints with the addition of cetuximab to FOLFIRI *vs.* FOLFIRI alone. Treatment with cetuximab offered significant benefit in those with wild-type *RAS* tumors (28.4 *vs.* 20.2 months with FOLFIRI alone).

In sum, these findings support a benefit from the addition of cetuximab or panitumumab to combination chemotherapy in the first-line treatment of patients with wild-type *KRAS* metastatic colorectal tumors. In contrast, the addition of anti-EGFR therapy in metastatic *KRAS*-mutant tumors (including extended *RAS* mutations) offers no benefit in this setting and has even been shown to be

detrimental. Cetuximab or panitumumab + FOLFOX and cetuximab + FOLFIRI are combinations of proven benefit in previously untreated wild-type *KRAS* mCRC. Nevertheless, a preferred backbone of chemotherapy has yet to be definitively proven in this setting.

Second-line settings

The EPIC study was an early phase III trial comparing irinotecan (350 mg/m² 90-minute infusion every 3 weeks) to cetuximab + irinotecan in 1,298 patients with EGFR-expressing mCRC who progressed on first-line fluoropyrimidine and oxaliplatin therapy (39). Although conducted prior to the establishment of *KRAS* as a predictive biomarker, the addition of cetuximab to irinotecan significantly improved PFS and ORR *vs.* irinotecan alone (Table 3). Median OS was comparable in both arms and the lack in difference may have been contributed by the 46.9% of patients randomized to irinotecan alone who eventually received cetuximab. Grade ≥3 AEs of diarrhea and acne-form rash were more frequent in the cetuximab + irinotecan group (28.4% and 8.2%, respectively) *vs.* the irinotecan alone group (15.7% and 0.2%, respectively).

The 20050181 trial, however, randomized 1,186 patients with mCRC to second-line FOLFIRI + panitumumab *vs.* FOLFIRI and prospectively analyzed efficacy by *KRAS* mutation status (40). Results from this phase III trial showed an improvement in PFS and ORR with FOLFIRI + panitumumab over FOLFIRI among wild-type *KRAS* tumors, but no benefits were observed in PFS and ORR with the addition of panitumumab among mutant *KRAS* tumors

(Table 3). In the *KRAS*-wild-type population, improved median OS was observed in favor of FOLFIRI + panitumumab (14.5 months) over FOLFIRI (12.5 months, $P=0.12$) though not statistically significant likely secondary to the 31% of patients in the FOLFIRI arm who subsequently received anti-EGFR therapy. Grade ≥ 3 skin toxicities, neutropenia, and diarrhea were the most frequent AEs in the FOLFIRI + panitumumab arms in both mutant and wild-type cohorts. In an updated analysis, no benefits were observed again with FOLFIRI + panitumumab compared to FOLFIRI in those with extended *RAS* mutations (42). Significant improvement in PFS was seen with FOLFIRI + panitumumab over FOLFIRI alone in those with wild-type *RAS* tumors (*KRAS* and *NRAS* exons 2, 3, and 4, HR 0.70, 95% CI, 0.54–0.91, $P=0.007$) and wild-type *RAS* and *BRAF* tumors (HR 0.68, 95% CI, 0.51–0.90, $P=0.006$).

The PICCOLO trial prospectively assigned 460 patients with wild-type *KRAS* mCRC (codons 12, 13, and 61) who progressed on first-line fluoropyrimidine-based chemotherapy to irinotecan + panitumumab (9 mg/kg IV every 3 weeks) or irinotecan alone (41). The addition of panitumumab to irinotecan offered advantages in PFS and ORR compared to irinotecan alone, but no significant difference was observed in OS between arms (Table 3). The lack of significant difference in OS cannot be attributed to cross over to anti-EGFR therapy in the irinotecan alone group as only 6% subsequently received anti-EGFR therapy. Instead, more rapid tumor growth upon stopping anti-EGFR therapy may have contributed to inferior survival in the irinotecan + panitumumab arm. Notably, interaction tests identified no benefit on PFS or ORR and a detrimental effect on OS with the addition of panitumumab in patients with any mutation present [*KRAS* (codons 12, 13, 61, and 146), *BRAF* (codon 600), *NRAS* (codons 12, 13, and 61), *PIK3CA* (exons 9 and 20)]. Toxicities were consistent to those seen in prior trials involving anti-EGFR monoclonal antibodies in combination with irinotecan.

The addition of an anti-EGFR agent to the second-line treatment of mCRC offers improvements in PFS and ORR that are limited to those with wild-type *KRAS* tumors. The lack of OS benefit observed may be attributed to the relatively high degree of cross over to anti-EGFR therapy in control arms and/or worse outcomes seen with anti-EGFR therapy withdrawal in the setting of high dose every-3-week irinotecan. Treatment with anti-EGFR therapy confers a lack of benefit to even harm in those who are *KRAS* mutant in this setting. As evident from the above, determination

of *RAS* mutation status is crucial in predicting benefit (or harm) from the addition of anti-EGFR therapy across all treatment settings in mCRC.

Anti-EGFR therapy versus bevacizumab in the first-line treatment of RAS-wild-type metastatic CRC

Several large RCTs have attempted to compare the benefits of adding anti-EGFR monoclonal antibodies or bevacizumab to the first-line treatment in patients with wild-type *RAS* mCRC (Table 4). The phase III FIRE-3 trial randomized 592 patients with wild-type *KRAS* (exon 2) mCRC to FOLFIRI + bevacizumab (5 mg/kg 90-minute infusion initially then over 60 minutes 2 weeks later followed by over 30 minutes every 2 weeks thereafter) *vs.* FOLFIRI + cetuximab (43). Significant differences in ORR and PFS were not observed between arms though OS was significantly improved with FOLFIRI + cetuximab over FOLFIRI + bevacizumab. Similar results were seen in those with wild-type tumors at other *RAS* loci though a marked advantage was observed in median OS with FOLFIRI + cetuximab (33.1 months) over FOLFIRI + bevacizumab (25.6 months; HR 0.70; 95% CI, 0.53–0.92; $P=0.011$). Hematologic toxicity and diarrhea were among the most common grade ≥ 3 AEs in both arms; skin reactions were more common with FOLFIRI + cetuximab. A subsequent independent radiological review of FIRE-3 did identify improved ORR with FOLFIRI + cetuximab (71.4%) over FOLFIRI + bevacizumab (56.4%, $P=0.015$), greater early tumor shrinkage with FOLFIRI + cetuximab (67.5% *vs.* 47.9%, $P=0.0013$), and greater depth of response with FOLFIRI + cetuximab (48.2% *vs.* 33.0%, $P=0.0005$) that may have all contributed to the discrepancy between OS and PFS across arms (47). However, the limited number of patients who crossed over to anti-EGFR therapy on progression in the bevacizumab-containing arm (41%) may also be contributing.

The PEAK trial was a phase II study investigating modified FOLFOX6 (mFOLFOX6) + panitumumab *vs.* mFOLFOX6 + bevacizumab in 285 treatment-naïve patients with wild-type *KRAS* (exon 2) metastatic colorectal tumors (44). ORR and PFS were similar between arms but OS was significantly improved with mFOLFOX6 + panitumumab over mFOLFOX6 + bevacizumab in the intent-to-treat wild-type *KRAS* exon 2 cohort (Table 4). In the extended wild-type *RAS* population, median PFS was significantly improved in favor of mFOLFOX6 + panitumumab (13.0 *vs.* 9.5 months; HR 0.65; 95% CI, 0.44–0.96; $P=0.029$) and an improvement in median

Table 4 Anti-EGFR therapy versus bevacizumab in the first-line treatment of RAS-wild-type metastatic colorectal cancer

Study	n=size	Arms	ORR	PFS	OS
FIRE-3 (43)	592	FOLFIRI + cetuximab vs. FOLFIRI + bevacizumab	62.0% vs. 58.0% (OR 1.18; 95% CI, 0.85–1.64; P=0.18)	Median 10.0 vs. 10.3 months (HR 1.06; 95% CI, 0.88–1.26; P=0.55)	Median 28.7 vs. 25.0 months (HR 0.77; 95% CI, 0.62–0.96; P=0.017)
PEAK (44)	285	mFOLFOX6 + panitumumab vs. mFOLFOX6 + bevacizumab	57.8% vs. 53.5% (P value NR)	Median 10.9 vs. 10.1 months (HR 0.87; 95% CI, 0.65–1.17; P=0.353)	Median 34.2 vs. 24.3 months (HR 0.62; 95% CI, 0.44–0.89; P=0.009)
CALGB 80405 (45,46)	1137	Chemotherapy (mFOLFOX6 or FOLFIRI) + cetuximab vs. chemotherapy + bevacizumab	65.6% vs. 57.2% (P value NR)	Median 10.4 vs. 10.8 months (HR 1.04; 95% CI, 0.91–1.17; P=0.55)	Median 29.9 vs. 29.0 months (HR 0.92; 95% CI, 0.78–1.09; P=0.34)

ORR, overall response rate; PFS, progression-free survival; OS, overall survival; FOLFIRI, 5-fluorouracil + leucovorin + irinotecan; OR, odds ratio; CI, confidence interval; HR, hazard ratio; mFOLFOX6, modified 5-fluorouracil + leucovorin + oxaliplatin; NR, not reported.

OS approached significance in favor of mFOLFOX6 + panitumumab [41.3 vs. 28.9 months (HR 0.63, 95% CI, 0.39–1.02, P=0.058)]. Skin toxicity and hypertension were the most common grade ≥ 3 AEs in the panitumumab- and bevacizumab-containing arms, respectively. Notably, the PEAK trial was limited by relatively small sample size and low cross over rate (38%) onto salvage anti-EGFR therapy on progression in the bevacizumab-containing arm.

The phase III CALGB/SWOG 80405 study randomized 1,137 patients with wild-type *KRAS* (codons 12 and 13) metastatic colorectal tumors to first-line standard chemotherapy (choice of FOLFIRI or mFOLFOX6) + cetuximab or bevacizumab (45). The majority of patients (73.4%) received mFOLFOX6, and no differences were observed in PFS and OS between arms (Table 4). In an updated analysis, chemotherapy + cetuximab demonstrated an improved ORR in the wild-type *KRAS* (exon 2) group (65.6% vs. 57.2%) and extended wild-type *RAS* group (68.6% vs. 53.6%) over chemotherapy + bevacizumab, but again there were no differences in PFS and OS (46). Of 132 patients who were able to undergo curative surgery following chemotherapy, 82 were in the cetuximab arm and 50 were in the bevacizumab arm (48). In this group who achieved curative resection, median OS was more than 5 years but was not significantly different between cetuximab- or bevacizumab-treated patients.

To sum, the FIRE-3, PEAK, and CALGB 80405 studies failed to meet their respective primary endpoints but mature data for depth of response, early tumor shrinkage, and impact of post-progression therapy are still awaited for CALGB 80405. There is preliminary evidence to support the use of anti-EGFR therapy in the first-line treatment of wild-type *RAS* mCRC over bevacizumab-containing chemotherapy

for patients with goals of tumor shrinkage for conversion to surgical resection or symptomatic disease. Nevertheless, an optimal treatment sequence in wild-type *RAS* mCRC cannot be definitively made with the current evidence, and the choice of first-line anti-EGFR- or bevacizumab-containing therapy should be made based on toxicity, cost, availability, and other parameters tailored to the patient.

KRAS mutant subtypes and impact on outcome

The RASCAL II study further expanded and explored the impact of various *KRAS* mutations on CRC outcome from the RASCAL study and identified that only those with a glycine to valine substitution on codon 12 (G12V) demonstrated significantly inferior disease-free survival (DFS) and OS in stage III but not stage IV CRC, when compared to wild-type controls, suggesting that *KRAS*^{G12V} mutations may predispose to a more aggressive phenotype (49). In mCRC, pooled analyses have shown that patients with *KRAS*^{G13D} mutated tumors may derive statistically significant benefits from the addition of cetuximab to chemotherapy when compared to other *KRAS* mutant subgroups in first-line and beyond settings (50,51).

However, in a recent pooled analysis of the 408, PRIME, and 20050181 studies, no *KRAS* mutant allele [out of the seven most common mutations evaluated in *KRAS* codons 12 and 13 (G12A, G12C, G12D, G12R, G12S, G12V, and G13D)] was consistently shown to be a prognostic biomarker for PFS or OS in patients with mCRC receiving panitumumab therapy (52). The phase II ICECREAM trial assessed the efficacy of cetuximab vs. cetuximab + irinotecan in mCRC patients stratified to *KRAS*^{G13D} mutation or no mutation (53). Of 51 patients so far enrolled with *KRAS*^{G13D} mutant tumors

Table 5 BRAF mutation status and anti-EGFR therapy in metastatic colorectal cancer

Study	Arms	Frequency of BRAF mutants	BRAF-mutant OS (median)	BRAF-wt OS (median)
First-line settings				
CAIRO2 (22,59)	XELOX + bevacizumab	45/519 (8.7%)	15.0 months	24.6 months
	XELOX + bevacizumab + cetuximab		15.2 months	21.5 months
COIN (32)	XELOX or FOLFOX	102/1,316 (7.8%)	10.0 months	20.1 months (KRAS/NRAS/BRAF-wt)
	XELOX or FOLFOX + cetuximab		7.2 months	19.9 months (KRAS/NRAS/BRAF-wt)
NORDIC VII (33)	FLOX +/- cetuximab	55/457 (12.0%)	9.5 months	22.0 months
CRYSTAL (36)	FOLFIRI	60/999 (6.0%)	10.3 months	21.6 months (KRAS/BRAF-wt)
	FOLFIRI + cetuximab		14.1 months	25.1 months (KRAS/BRAF-wt)
PRIME post hoc analysis (35)	FOLFOX4	53/619 (9.0%)	9.2 months	15.8 months
	FOLFOX4 + panitumumab		10.5 months	14.5 months
Second-line settings				
20050181 post hoc analysis (42)	FOLFIRI	45/541 (8.3%)	5.7 months	15.4 months (RAS/BRAF-wt)
	FOLFIRI + panitumumab		4.7 months	18.7 months (RAS/BRAF-wt)
PICCOLO (41)	Irinotecan	68/460 (14.8%)	HR 1.56 (95% CI, 1.03–2.37; P=0.035) of BRAF-mutant vs. KRAS/NRAS/BRAF-wt	
	Irinotecan + panitumumab		HR 1.84 (95% CI, 1.10–3.08; P=0.029) of BRAF-mutant vs. KRAS/NRAS/BRAF-wt	

OS, overall survival; wt, wild-type; XELOX, capecitabine + oxaliplatin; FOLFOX, 5-fluorouracil + leucovorin + oxaliplatin; FLOX, bolus 5-fluorouracil + leucovorin + oxaliplatin; FOLFIRI, 5-fluorouracil + leucovorin + irinotecan; HR, hazard ratio; CI, confidence interval.

refractory to irinotecan- or oxaliplatin-based chemotherapy, median time to progression (TTP) was comparable between arms but stable disease (SD) was achieved in 58% of patients treated with cetuximab and 70% with cetuximab + irinotecan. In short, there remains a lack of convincing evidence to argue that anti-EGFR therapy should not be limited to patients with wild-type *KRAS* mCRC.

BRAF mutation status as a biomarker of response to anti-EGFR therapy

In a pooled analysis of RCTs, the prevalence of *BRAF* mutations in patients with mCRC has been shown to be 8.1% (18). Oncogenic *BRAF* mutations, of which the majority are codon V600E, have been associated with microsatellite instability (MSI), multiple sites of metastases, colon (in particular right-sided) rather than rectal tumors, high-grade tumors, mucinous histology, adverse histologic features, older age, Eastern Cooperative Oncology Group (ECOG) performance status (PS) ≥ 2 , female gender, and poor survival [especially those with microsatellite stable (MSS) tumors] in mCRC (18,54-58). In several mutational analyses, *BRAF* mutations have been identified

as independent factors significantly associated with reduced survival in mCRC patients (57,58). Similar to *RAS* mutations, *BRAF* mutation status appears to be a predictor of benefit (or lack of) from anti-EGFR monoclonal antibodies, cetuximab or panitumumab, in first-line and beyond treatment settings in mCRC.

First-line and beyond settings

Early retrospective studies were among the first to identify the lack of benefit to anti-EGFR in those with *BRAF*-mutated metastatic colorectal tumors. In one study, 6 of 48 patients (12.5%) with *BRAF*-mutant mCRC were treated with cetuximab or panitumumab in the first-line to fourth-line settings, and none demonstrated an objective response (14). Similarly, out of 79 patients who were *KRAS*-wild-type, there were no responses seen in the 11 patients who had *BRAF* mutations treated with cetuximab or panitumumab for their chemorefractory mCRC (15). The addition of anti-EGFR therapy to the first-line or second-line treatment of mCRC has consistently demonstrated a lack of benefit in those with *BRAF*-mutant tumors compared to their wild-type counterparts (Table 5). These

Table 6 Targeting the RAS/RAF/MAPK pathway in BRAF-mutant metastatic colorectal cancer

Study	n= size	Arm (s)	ORR	PFS	OS
Kopetz <i>et al.</i> (62)	21	Vemurafenib	5%	Median 2.1 months	Median 7.7 months
Falchook <i>et al.</i> (63)	9	Dabrafenib	11.1%	NR	NR
Infante <i>et al.</i> (64)	28 (KRAS- or BRAF-mutant)	Trametinib	0%	NR	NR
Hong <i>et al.</i> (65)	17	Vemurafenib + cetuximab + irinotecan	35%	Median 7.7 months	NR
Hyman <i>et al.</i> (66)	27	Vemurafenib + cetuximab	4% (95% CI, <1–20)	Median 3.7 months (95% CI, 1.8–5.1)	Median 7.1 months (95% CI, 4.4–not reached)
Tabernero <i>et al.</i> (67)	14	Vemurafenib + cetuximab	SD 60% at week 8, SD 66.6% at week 16	NR	NR
Yaeger <i>et al.</i> (68)	15	Vemurafenib + panitumumab	16.7%	Median 3.2 months (95% CI, 1.6–5.3)	Median OS 7.6 months (95% CI, 2.1–not reached)
Corcoran <i>et al.</i> (69)	43	Dabrafenib + trametinib	12%	Median 3.5 months (95% CI, 3.4–4.0)	NR
Schellens <i>et al.</i> (70)	54	Encorafenib + cetuximab (A); encorafenib + cetuximab + alpelisib (B)	A: 23%; B: 32%	A: median 3.7 months; B: median 4.3 months	NR
Van Cutsem <i>et al.</i> (71)	55	Dabrafenib + panitumumab (D + P); dabrafenib + trametinib + panitumumab (D + T + P)	D + P: 10% (95% CI, 1.2–31.7); D + T + P: 26% (95% CI, 12.5–43.3)	D + P: median 3.5 months (95% CI, 2.8–5.8); D + T + P: median 4.1 months (95% CI, 2.6–5.5)	NR

ORR, overall response rate; PFS, progression-free survival; OS, overall survival; NR, not reported; CI, confidence interval; SD, stable disease.

findings have been observed across several phase III trials (22,32,33,36,37,41,42,59). Indeed, a recent meta-analysis of phase II and III trials confirmed that the addition of anti-EGFR therapy to standard chemotherapy or BSC did not significantly improve PFS, OS, or ORR in those with *BRAF*-mutant mCRC, when compared to control regimens (60). Notably, addition of cetuximab to FOLFIRI in the CRYSTAL study showed a trend towards benefit in PFS and OS (significance was not reached) compared to FOLFIRI alone in those with wild-type *KRAS* but *BRAF*-mutant tumors (36). A pooled analysis of the OPUS and CRYSTAL studies also showed a trend towards benefit in PFS and OS (significance not reached) with addition of cetuximab to chemotherapy compared to chemotherapy alone in wild-type *KRAS* but *BRAF*-mutant tumors (61). These data, in aggregate, suggest that there is limited activity for anti-EGFR therapy in metastatic *BRAF*-mutant colorectal tumors. Subsequent investigations have focused on circumventing resistance to EGFR inhibition associated with *BRAF* mutations in mCRC.

Targeting additional mediators of the RAS/RAF/MAPK pathway

Aside from targeting EGFR, an initial phase II study investigated vemurafenib, an oral *BRAF*^{V600E} inhibitor, in 21 patients with predominantly chemorefractory and *BRAF*^{V600E}-mutant mCRC at 960 mg twice daily (62). Single-agent vemurafenib did not show meaningful activity in this population (Table 6). The most common grade ≥ 3 AEs were squamous cell carcinoma (SCC) of the skin, hyperbilirubinemia, rash, and hyponatremia. Similarly, in a phase I dose-escalation study where the majority of patients were treated with a recommended phase II dose of the oral *BRAF* inhibitor dabrafenib (150 mg twice daily), only 1 of 9 evaluable patients (11.1%) with treatment-naïve or refractory *BRAF*-mutant mCRC experienced an objective response (63). The most frequent grade ≥ 2 AEs were cutaneous SCC or keratoacanthoma, fatigue, and pyrexia. Another phase I dose-escalation study investigating the oral MEK1/MEK2 inhibitor trametinib [majority at 2 mg daily for 21 days (every 28 days)] failed to identify an objective

response in 28 patients with treatment-refractory *KRAS*- or *BRAF*-mutant advanced CRC (64). The most common AEs of the overall cohort were dermatitis acneiform (80%) and diarrhea (42%). The limited activity seen with single-agent BRAF and EGFR inhibitors in *BRAF*-mutant mCRC has been better elucidated with further understanding of feedback loops along the RAS/RAF/MAPK pathway.

Constitutive activation of BRAF^{V600E} promotes cell proliferation, growth, and survival through MEK/ERK signaling yet suppresses stimulation from related pathways including RAS-induced activation of RAF isoforms (CRAF and ARAF) through negative feedback (72). Inhibition of BRAF^{V600E} results in loss of negative feedback and promotion of RAS/RAF signaling. Other mediators of BRAF-inhibitor resistance include EGFR activation, mutations in MEK itself, *BRAF*^{V600E} amplifications, and *MAP3K8/COT* mutations. Furthermore, BRAF inhibitor-induced feedback activation of EGFR appears to be dependent, in part, by inhibition of the phosphatase CDC25C (73). MEK inhibition, like BRAF inhibition, has also been shown to activate EGFR through feedback regulation (73). In an attempt to overcome the various mechanisms of MAPK pathway-inhibitor resistance, preclinical studies have demonstrated improved antitumor efficacy with combination EGFR, BRAF, or MEK inhibition in *BRAF*-mutant CRC (73,74). Preclinical studies such as these have essentially established the framework for subsequent clinical trials in *BRAF*-mutant mCRC treatment.

One phase Ib study attempted to address the compensatory activation of EGFR with BRAF blockade by adding cetuximab to vemurafenib and irinotecan in 17 patients with *BRAF*-mutant mCRC (Table 6). An improvement in ORR was produced with a median duration of response of 12.5 cycles (65). The maximum tolerated dose (MTD) determined was vemurafenib 960 mg twice daily + cetuximab 250 mg/m² weekly + irinotecan 180 mg/m² every 14 days. Fatigue (94%), diarrhea (89%), nausea (83%), and rash (78%) were among the most common AEs. The phase II SWOG 1406 study will build on these findings and investigate irinotecan (180 mg/m² IV every 2 weeks) + cetuximab (500 mg/m² IV every 2 weeks) ± vemurafenib (960 mg oral twice daily) in patients with *BRAF*^{V600E}-mutant mCRC who have progressed on first- or second-line therapies (75). Additionally, several other early phase clinical trials have explored the combination of vemurafenib + EGFR inhibitor and demonstrated modest activity in

patients with chemorefractory metastatic *BRAF*-mutated colorectal tumors (66–68). Notably, data from these trials are preliminary with OS not reached thus rendering definitive conclusions difficult to make until maturation or evaluation in larger trials (Table 6). Interestingly, the incidence of grade ≥3 dermatologic toxicity was lower in combination BRAF + EGFR inhibitor arms than in single-agent BRAF or EGFR inhibitor arms, but abnormal liver function tests were more common in the combination arms (66,68).

Alternatively, combined BRAF and MEK inhibition with dabrafenib (150 mg twice daily) + trametinib (2 mg daily), respectively, demonstrated activity in 43 patients with predominantly pretreated mCRC bearing *BRAF*^{V600E} mutations (Table 6). Anemia, pyrexia, fatigue, and vomiting were among the most frequent grade ≥3 AEs (69). A phase I study investigated the highly selective oral BRAF^{V600E} inhibitor encorafenib + cetuximab ± the alpha-specific PI3K inhibitor alpelisib in 54 patients with mutated *BRAF* mCRC (70). The triple combination arm had an improved ORR and PFS than the encorafenib + cetuximab arm though statistical differences, if any, have yet to be reported (Table 6). The most common grade ≥3 AEs included fatigue, hypophosphatemia, and nausea and a MTD of encorafenib 200 mg daily + cetuximab 250 mg/m² IV weekly ± alpelisib 300 mg daily was proposed. Combinations of dabrafenib (150 mg twice daily) + panitumumab (6 mg/kg IV every 2 weeks) or dabrafenib + trametinib (2 mg daily) + panitumumab were used in the treatment of 55 patients with *BRAF*-mutant mCRC (71). The triple combination arm demonstrated greater improvement in endpoints as well as more profound reduction in pERK levels on biopsies taken at day 15 compared to the dual combination arm (Table 6). The most common AEs were dermatitis acneiform and diarrhea (triple combination arm) and dermatitis acneiform and fatigue (dual combination arm).

In sum, there is growing evidence to suggest that there is a lack of clinically meaningful benefit with anti-EGFR therapy (single-agent or in combination with standard chemotherapy) in those with *BRAF*-mutant mCRC. Similarly, single-agent BRAF and MEK inhibitors appear to have limited activity in this group likely owing to mechanisms of resistance/feedback regulation intrinsic to the RAS/RAF/MAPK signaling pathway. Combined inhibition of targets such as EGFR, BRAF, and/or MEK is showing promising activity in *BRAF*-mutant mCRC compared to historical controls involving single targeting.

***BRAF*-mutant metastatic CRC and first-line therapy**

In an initial phase II study investigating first-line irinotecan 165 mg/m² IV on day 1 + oxaliplatin 85 mg/m² IV on day 1 + LV 200 mg/m² IV on day 1 + 5-FU 3,200 mg/m² 48-hour infusion over days 1–2 (FOLFOXIRI) + bevacizumab every 2 weeks, 214 eligible patients with mCRC were screened for BRAF mutational status (76). Fifteen patients with *BRAF*-mutant tumors were included in the validation cohort and demonstrated an ORR of 72%, median PFS of 9.2 (95% CI, 5.1–13.3), and median OS of 24.1 months (95% CI, 3.3–45.0). The subsequent phase III TRIBE study randomized 508 patients with mCRC to FOLFOXIRI + bevacizumab *vs.* FOLFIRI + bevacizumab (every 2 weeks up to 12 cycles then transitioned to maintenance 5-FU/LV + bevacizumab) in the first-line setting (77). FOLFOXIRI + bevacizumab demonstrated superior PFS and OS (median, 29.8 months) over FOLFIRI + bevacizumab [25.8 months (HR 0.80; 95% CI, 0.65–0.98; P=0.03)] in the overall intent-to-treat population. In the *BRAF*-mutant and extended *RAS*-mutant subgroups, FOLFOXIRI + bevacizumab also showed improved PFS and OS over FOLFIRI + bevacizumab. Grade ≥ 3 neutropenia, diarrhea, and asthenia were among the most common AEs in the FOLFOXIRI + bevacizumab arm and were more frequent than in the FOLFIRI + bevacizumab arm. Mutant *BRAF* status portends an aggressive phenotype and poor prognosis in mCRC. Given the benefits offered by FOLFOXIRI + bevacizumab irrespective of *RAS* or *BRAF* mutational status, this combination should be considered in the first-line treatment of *BRAF*-mutant mCRC.

Discussion and future directions

The management of mCRC with respect to *RAS* and *BRAF* mutational status has seen much change over the past decade or so. In 2006, the American Society of Clinical Oncology (ASCO) recommended against routine *RAS* mutational testing in mCRC due to insufficient and heterogeneous data at the time (78). In 2009, ASCO released a provisional clinical opinion based on available data and recommended routine testing for *KRAS* mutations (codons 12 and 13) for selection of candidates for treatment with cetuximab and panitumumab in mCRC (79). The National Comprehensive Cancer Network (NCCN) now mandates routine testing for *KRAS* exon 2 and extended *RAS* (*KRAS* non-exon 2 and *NRAS*) mutations at diagnosis of mCRC; patients with any of these mutations should not be treated with anti-EGFR

therapy (80). Furthermore, the panel currently recommends for *BRAF*^{V600E} mutation testing at diagnosis of stage IV CRC as this mutation has significant prognostic implications that may impact patient counseling and treatment.

The ability to maximize the benefits offered by anti-EGFR therapy in wild-type *RAS* and *BRAF* mCRC at present and in the future will likely center around several key issues. Firstly, optimal selection of patients with mCRC who can benefit from anti-EGFR monoclonal antibodies depends on the performance of available molecular analysis technologies in detecting mutations. In an early investigation, 16 *KRAS* mutations were identified out of 59 patients with mCRC (27%) with traditional direct sequencing analysis (81). The use of more sensitive polymerase chain reaction (PCR)-based techniques detected another 6 *KRAS* mutations missed by direct sequencing that ultimately led to the detection of 22 mutations (37%) in total. Sanger sequencing has been characterized by poor sensitivity that requires *KRAS* mutant alleles to represent $\geq 20\%$ of the signal in order to reliably detect the presence of a mutation (82). In contrast, pyrosequencing or quantitative PCR (qPCR) has greater sensitivity in detecting lower frequency mutations ranging from 1–10% of *KRAS* mutant DNA in a background of wild-type DNA (83). The significance of low frequency mutations (defined as mutant:wild-type *RAS* DNA ratio $\leq 10\%$) has been shown in patients with mCRC carrying low frequency *KRAS* mutations that amounted to a significantly inferior ORR and PFS with anti-EGFR therapy compared to wild-type *KRAS* patients (82). Digital PCR has shown even greater sensitivity with the ability to detect mutant *KRAS* alleles representing as low as 0.01% of the signal (83). Notably, mCRC patients with mutant:wild-type *KRAS* DNA ratios less than 1% have similar PFS and OS compared to wild-type *KRAS* patients treated with anti-EGFR therapy (83). Future studies will need to focus on developing standardized and sensitive techniques to guide clinical decision making in selecting optimal candidates with mCRC for anti-EGFR therapy. The development of next-generation sequencing (NGS) with high-throughput functionality, which has shown comparable accuracy in detecting *RAS* mutations to real-time PCR, represents one increasingly popular strategy (84).

The optimal selection of mCRC patients who can benefit from anti-EGFR therapy is also dependent on identification of additional markers of resistance in those with wild-type *RAS* and *BRAF* tumors. Much progress has been made in understanding that extended *RAS* mutations including *NRAS* and non-exon 2 *KRAS* mutations are predictive of absence

of benefit to EGFR blockade. *RAS* and *BRAF* mutations are now found in approximately 50–55% of cases of mCRC. Preclinical studies have identified that colorectal tumors harboring amplifications in *KRAS*, although infrequent (<1%), are not responsive to cetuximab or panitumumab (85). CpG island methylator phenotype (CIMP)-high methylation status ($\geq 40\%$) has been significantly associated with reduced PFS in wild-type *RAS* and *BRAF* mCRC patients treated with first-line anti-EGFR therapy (86). *ERRB2* (*HER2/neu*) and *MET* amplifications have also been shown to promote resistance to anti-EGFR monoclonal antibodies in colorectal tumors (87,88). Similarly mutations along the PTEN/PIK3CA/AKT signaling pathway have been associated with lack of response to anti-EGFR therapy in wild-type *KRAS* mCRC (89). *PIK3CA* mutations (exon 20) have also been associated with worse outcomes in wild-type *KRAS* mCRC treated with cetuximab (90). The Cancer Genome Atlas (TCGA) Network previously identified gene alterations in *BRAF*, *ARAF*, *CRAF*, and *NRAS* of very low frequency (<1% of cases) in their CRC dataset whose association with response to anti-EGFR therapy in wild-type *RAS* and *BRAF* metastatic colorectal tumors has yet to be elucidated (91). Recent genomic analyses have identified mutations in *EGFR* (including alterations in the ectodomain of EGFR), *FGFR1*, *PDGFRA*, and *MAP2K1* as additional potential mediators of resistance to anti-EGFR therapy in wild-type *KRAS* mCRC (92). Interestingly, alterations in the tyrosine kinase receptor adaptor gene *ISR2* may be associated with increased sensitivity to anti-EGFR agents (92). The list of potential markers of anti-EGFR resistance in wild-type *RAS* and *BRAF* mCRC continues to grow. Comprehensive genomic analyses via NGS, with its massive throughput capabilities, will likely continue to play a major role in facilitating the search for additional predictors of resistance that were previously undetectable due to feasibility and technical reasons.

A third issue that is important in maximizing the efficacy of anti-EGFR monoclonal antibodies in wild-type *RAS* and *BRAF* mCRC involves overcoming mechanisms of resistance. The mechanisms of acquired or secondary resistance to EGFR inhibition has been broadly characterized by mutations that disrupt binding of cetuximab or panitumumab to the EGFR, pathway mutations that bypass the site of inhibition (alterations in *KRAS* or *BRAF*), and mutations along parallel pathways (*MET* or *HER2*) (93). With the exception of the EGFR extracellular domain mutation S492R, mechanisms of primary resistance overlap with those of secondary resistance. More and more gene alterations are being

identified as potential biomarkers of response to EGFR inhibition in wild-type tumors, yet it has been postulated that the majority of alterations will converge to activate the EGFR/RAS/MAPK pathway as evidenced by the frequency of mutations involving mediators of EGFR signaling (*EGFR*, *RAS*, *BRAF*, and *PIK3CA*). In order to improve the efficacy of anti-EGFR therapy in wild-type *RAS* and *BRAF* tumors, future studies will need to focus on the centrality of MAPK signaling in driving resistance.

Preclinical studies have recently demonstrated synergistic antitumor activity in *BRAF*-mutant CRC cells with addition of PI3K inhibitors or methyltransferase to vemurafenib (94). Similarly, greater tumor regression was seen in *BRAF*-mutant CRC cells treated with combined BRAF and dual PI3K/mTOR inhibition (95). In *BRAF*-mutant mCRC patients treated with RAF + EGFR inhibition or RAF + MEK inhibition, acquired resistance was characterized by development of a *KRAS* amplification, *BRAF* amplification, *ARAF* mutation, or *MEK1* mutation (96). Addition of an ERK inhibitor was able to suppress MAPK activity and overcome resistance in this group. The growing number of clinical trials investigating combined EGFR, BRAF, and/or MEK inhibition that have shown promising activity in *BRAF*-mutant mCRC highlight the importance of inhibiting multiple steps along the EGFR signaling cascade to enhance activity. Future trials with combination therapy in both *RAS*- and *BRAF*-mutant mCRC are likely to increase in number with the goal to improve therapeutic efficacy through sustained MAPK pathway inhibition.

Lastly, recent analysis of the FIRE-3 trial demonstrated significant improvement in ORR, PFS, and OS with cetuximab therapy in patients with wild-type *KRAS* metastatic tumors arising from the left colon compared to right colon (97). Similarly, analysis of the CO.17 trial identified that wild-type *KRAS* mCRC patients with primary tumors located to the left colon experienced significantly improved PFS when treated with cetuximab *vs.* BSC (median 5.4 *vs.* 1.8 months; HR 0.28; 95% CI, 0.18–0.45; $P < 0.0001$) while those with primaries located to the right colon did not (97). Median PFS in those with wild-type *KRAS* metastatic tumors of right colon primary treated with cetuximab was 1.9 *vs.* 1.9 months in those treated with BSC (HR 0.73; 95% CI, 0.42–1.27; $P = 0.26$). In wild-type *KRAS* mCRC, there is growing evidence to suggest that tumor location is also predictive of benefit to anti-EGFR therapy. Further investigation is duly warranted to explore the interactions between primary right-sided and left-sided colorectal tumors and response to anti-EGFR therapy in

the wild-type *RAS* and *BRAF* metastatic population.

Conclusions

All patients with mCRC should undergo *RAS* (*KRAS* and *NRAS*) and *BRAF* mutational analysis at the time of diagnosis. Treatment with anti-EGFR monoclonal antibodies (cetuximab or panitumumab), either as single-agent or combination therapy, offers improved outcomes in wild-type *RAS* mCRC. The optimal sequencing of EGFR inhibitors and bevacizumab has yet to be defined. The incorporation of anti-EGFR treatment in the continuum of treatment of metastatic *RAS* wild-type colon cancer is acceptable in the first, second, or subsequent lines of treatment. There is a lack of benefit and even harm with anti-EGFR therapy in *RAS*-mutant mCRC. Similarly, *BRAF* mutations have been shown to predict resistance to anti-EGFR therapy in mCRC. Clinical trials involving combinations of BRAF, EGFR, and/or MEK inhibitors have shown promising activity in *BRAF*-mutant mCRC. Comprehensive genomic profiling via NGS can facilitate the identification of additional markers of resistance to EGFR inhibition in wild-type *RAS* and *BRAF* metastatic colorectal tumors. Future studies will likely focus on combined inhibition of multiple targets along the MAPK signaling pathway to overcome resistance to anti-EGFR therapy in this population.

Acknowledgements

None.

Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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Cite this article as: Gong J, Cho M, Fakhri M. RAS and BRAF in metastatic colorectal cancer management. *J Gastrointest Oncol* 2016;7(5):687-704. doi: 10.21037/jgo.2016.06.12