Profile of panitumumab as first-line treatment in patients with wild-type KRAS metastatic colorectal cancer

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Abstract: Targeted therapies against EGFR, vascular endothelial growth factor, and vascular endothelial growth factor receptor have expanded treatment options for patients with metastatic colorectal cancer (mCRC). Unfortunately, biomarkers to identify patients that are most likely to derive benefit from targeted therapies in this disease are still needed. Indeed, only RAS mutations have been identified as predictive of lack of benefit from monoclonal antibodies against EGFR in patients with mCRC. Panitumumab is a fully humanized monoclonal antibody against EGFR. In this study, we review data to support the use of panitumumab in combination with a chemotherapy backbone, in the first line setting in patients with RAS wild-type mCRC. Ongoing efforts are aimed at identifying smaller subsets of patients within the RAS wild-type group that will derive the largest benefit from anti-EGFR therapy. In the meantime, treatment with anti-EGFR therapy should be reserved for patients with RAS wild-type mCRC.

Keywords: panitumumab, metastatic colorectal cancer, first-line, RAS

Introduction

Colorectal cancer (CRC) is the second most common cause of cancer death in the United States and Europe,1,2 although its incidence is steadily decreasing in the US.3 This is likely secondary to increased screening (colonoscopy) and resection of premalignant lesions (adenomatous polyps).4 Both in the US and Europe, survival is also steadily increasing.3,5 Palliative chemotherapy is the backbone of treatment for patients with unresectable metastatic colorectal cancer (mCRC). In these patients, 5-fluorouracil or its derivatives have been the standard cytotoxic treatment for nearly 50 years.6 Modulated 5-fluorouracil resulted in improved response rate (RR) with modest increments in survival. In the last decade, the addition of new cytotoxic agents (oxaliplatin, irinotecan) and, more recently, targeted therapies such as antiangiogenic agents (bevacizumab, aflibercept, regorafenib) or monoclonal antibodies against EGFR (cetuximab or panitumumab) have contributed to improving the outcomes of patients with newly diagnosed unresectable mCRC. Currently, treatment strategies for these patients are based on a continuum care paradigm whereby patients are exposed throughout the course of their disease to different active drugs, their treatment is personalized according to the need for rapid response and the burden of disease and RAS status, drugs are often reintroduced if they showed activity in a previous line of therapy, and finally, intervals of maintenance chemotherapy are considered.7 This strategy has recently provided survival figures above 30 months for patients with unresectable disease.8,9 Here, we review available data for the use of panitumumab, a monoclonal antibody against EGFR, as the first-line treatment in patients with KRAS exon 2 wild-type mCRC.
Epithelial growth factor signaling pathway in CRC

The EGFR family, or ErbB family, includes transmembrane glycoproteins with an intracellular tyrosine kinase domain, a transmembrane domain, and an extracellular ligand-binding domain. There are four transmembrane receptors in this family: HER1 (EGFR), HER2 (ErbB2), HER3 (ErbB3), and HER4 (ErbB4). These receptors can form homo- or heterodimers once activated. HER3 is the only member of this family that lacks a functional kinase domain and, therefore, can only be activated by forming heterodimers.

EGFR was first identified in 1978 in an A431 squamous cell carcinoma cell line. In this A431 cell line, EGF binding resulted in phosphorylation and activation of the receptor. EGFR has multiple domains (I–IV) (Figure 1). In its unbound form, EGFR adopts a tethered conformation that prevents its activation. When the tethered conformation is broken, EGFR ligands can bind domain III. This leads to stabilization of the receptor in its extending conformation, which exposes domain II, allowing the receptor to dimerize and initiate downstream signaling (Figure 1). Once activated, EGFR will form hetero- or homodimers and activate downstream signaling pathways including MAPK or the PI3K/mTOR pathway, leading to cancer cell proliferation, angiogenesis, migration, and survival.

The EGFR pathway can be deregulated at different levels resulting in increased EGFR ligands, increased EGFR expression and activating EGFR mutations. Activation of EGFR may result from binding to different ligands, including EGF, transforming growth factor α (TGF-α), amphiregulin, and heparin-binding EGF. EGFR expression in CRC ranges between 20% and 80%. However, a correlation between increased EGFR expression and response to monoclonal antibodies against EGFR has not been evidenced in CRC.

Figure 1 Schematic of EGFR with I, II, III, and IV representing extracellular domains.

Notes: (A) Represents tethered and untethered nonligand bound monomer conformations of EGFR. While in the untethered conformation, EGFR is able to be bound by GF ligands at domains I and III and obtain a more stabilized conformation (B) that allows for dimerization via domain II and downstream TK activation represented in (C).

(D) Cetuximab and panitumumab bind domain III of EGFR preventing untethering and subsequent ligand binding to domain I and III. Accordingly dimerization and TK activation is prevented.

Abbreviations: GF, growth factor; TK, tyrosine kinase.
patients with advanced CRC.\textsuperscript{21,22} Aberrations at the gene level involving \textit{EGFR} have also been reported in CRC. A smaller subset of CRC patients (8\%–12\%) have \textit{EGFR} amplifications defined as >5 gene copies/nucleus.\textsuperscript{23} A search of The Cancer Genome Atlas (TCGA) data from the cBioPortal for Cancer Genomics (\url{www.cbiportal.org}), data accessed on March 30, 2015 identified \textit{EGFR} missense mutations in 8 (3.7\%) patients with CRC (n=212). In addition, \textit{EGFR} was amplified in one patient (0.4\%). A similar search of COSMIC SANGER (\url{www.cancer.sanger.ac.uk/cancergenome}, data accessed on March 30, 2015) found \textit{EGFR} mutations present in 96 (7\%) of 1,294 tested samples. Early data suggested that increased \textit{EGFR} copy number, evaluated by fluorescence in situ hybridization, could predict response to \textit{EGFR} inhibitors in CRC.\textsuperscript{24,25} However, results from additional studies have been inconsistent and fail to confirm that hypothesis. In addition, a reproducible cut-off level of amplification that predicts response to anti-\textit{EGFR} therapy has not been identified in this disease.\textsuperscript{26}

Two monoclonal antibodies against \textit{EGFR} have gained regulatory approval for treating mCRC. Cetuximab was the first targeted therapy to gain approval in mCRC. Cetuximab is a chimeric IgG1 immunoglobulin, which binds \textit{EGFR} with high affinity. In cetuximab, the antigen-binding regions (Fv) of mouse antibody are combined with human IgG constant domains, which can lead to infusion reactions in up to 5\% of patients.\textsuperscript{27} According to the cetuximab label, premedication with antihistaminic drugs is recommended with the first infusion.\textsuperscript{28} Panitumumab, unlike cetuximab, is a fully humanized IgG2 monoclonal antibody. It was generated in transgenic strains of mouse and modified to express human immunoglobulin genes (XenoMouse). Panitumumab was developed by immunizing the XenoMouse IgG2 strain with cells from the human cervical epidermal carcinoma cell line A431. Neutralizing antibodies to this cell line were isolated and sorted by binding affinity to \textit{EGFR}.\textsuperscript{29} In vivo studies in xenografts from the A431 cell line showed that panitumumab blocked activation of \textit{EGFR}, prevented tumor formation, and eradicated established tumors.\textsuperscript{30} Since panitumumab is fully humanized, premedication is not recommended per US Food and Drug Administration (FDA) label since infusion reactions rarely occur (1\%–5\%).\textsuperscript{31} Similar to cetuximab, proposed mechanisms of growth inhibition include induction of cell cycle arrest, promotion of apoptosis, and \textit{EGFR} downregulation.\textsuperscript{32}

\textbf{Safety data}

The safety of panitumumab has been evaluated in different dose escalation studies. In the first study, four different dose levels of panitumumab (from 1 to 2.5 mg/kg weekly) were evaluated in 88 patients with advanced kidney cancer.\textsuperscript{33} Panitumumab was well tolerated in these patients. The most common adverse event (AE) was rash, which was usually most severe between the 3rd and the 5th week. At a 2.5 mg/kg weekly dose, 100\% of patients developed skin toxicity. The rash was typically distributed in the facial, periorificial regions, and the upper trunk. For most patients, rash intensity decreased despite treatment continuation. Responses were rare, but there was a trend toward improved progression-free survival (PFS) in patients with severe rash. No human antibodies were identified. A Phase I trial tested different schedules of panitumumab including: weekly panitumumab from 0.01 to 5 mg/kg, 6 mg/kg every 2 weeks, and 9 mg/kg every 3 weeks in 96 patients with different tumor types, most notably CRC (41\%).\textsuperscript{34} Treatment was well tolerated. The most common treatment-related AEs were rash (60\%) and dry skin (19\%). Most skin-related toxicities were grade 1–2. Seven percent of patients across all cohorts developed grade 3 skin toxicity (17\% in the every 3-week cohort). Median time for skin toxicity onset of any grade was 1 week. The maximum tolerated dose was not identified. Two patients in the 1 mg/kg weekly cohort had dose-limiting toxicities of severe maculopapular rash. One patient, at 2.5 mg/kg weekly, had a dose-limiting toxicity of fatigue, angina pectoris, and dyspnea. None of the patients experienced infusion reactions. Responses were seen in 13\% of patients with CRC. In addition, 23\% of CRC patients had a best response of stable disease. Drug exposure was similar with weekly doses at 2.5 mg/kg compared to 6 mg/kg every 2 weeks or 9 mg/kg every 3 weeks. A different Phase I study tested a dose of 6 mg/kg every 2 weeks given as a 60-minute infusion, or a 30-minute infusion if the first dose was well tolerated, or 9 mg/kg every 3 weeks as a 60-minute infusion.\textsuperscript{35} The study enrolled 84 patients with solid tumors (CRC =10). The incidence of grade 3 AEs was higher in the 9 mg q3 week cohort (62\% vs 37\%) although overall, the safety profile was similar across different schedules. Partial responses were seen in patients with CRC (18\%). In addition, 45\% of CRC patients attained stable disease. These initial studies showing preliminary evidence of activity in patients with CRC fueled interest in developing panitumumab for this disease.

\textbf{Panitumumab and oxaliplatin schedules in first line mCRC}

The PRIME study (Panitumumab Randomized trial In combination with chemotherapy for Metastatic colorectal cancer to determine Efficacy) showed improved PFS when panitumumab was added to FOLFOX (folinic acid,
5-fluorouracil, oxaliplatin) in the first-line treatment in exon 2 wild-type \textit{KRAS} mCRC. This randomized Phase III study allocated 1,183 patients in a 1:1 fashion to FOLFOX4 plus panitumumab (6 mg/kg every 2 weeks) vs FOLFOX4 alone. The study was initially designed to test treatment effect in all randomized patients. However, once \textit{KRAS} was established as a predictive biomarker, the study was amended to compare outcomes according to \textit{KRAS} status. \textit{KRAS} status was available for 93\% of the patients enrolled in the study.\textsuperscript{36} The primary endpoint was PFS. The final analysis, 30 months after the last patient was enrolled, showed improved PFS for exon 2 wild-type \textit{KRAS} patients enrolled in the FOLFOX4 plus panitumumab arm (10 vs 8.6 months, hazard ratio [HR] =0.80; 95\% confidence interval [CI]: 0.67–0.95, \textit{P}=0.01) (Table 1). A detrimental effect in PFS was seen in the subgroup of patients with exon 2 mutations enrolled in the FOLFOX4 plus panitumumab arm (7.4 vs 9.2 months, HR =1.27, 95\% CI: 1.04–1.55, \textit{P}=0.02). An exploratory analysis conducted after \textit{>80\%} patients had an overall survival (OS) event also showed an improvement in OS in the experimental arm (23.8 vs 19.4 months, HR =0.83, 95\% CI: 0.70–0.98, \textit{P}=0.03) in exon 2 wild-type \textit{KRAS} patients. Adding panitumumab also increased RR in exon 2 wild-type \textit{KRAS} patients (57\% vs 48\%, odds ratio =1.47, 95\% CI: 1.07–2.04, \textit{P}=0.02). However, no differences in complete resection rate were seen in exon 2 wild-type \textit{KRAS} patients with the addition of panitumumab (10\% vs 8\%). A subset analysis, limited to patients with liver-only disease, showed an increased rate of complete resection for patients in the FOLFOX plus panitumumab arm, but it was not statistically significant (27.9 vs 17.5, difference in rates =10.3, 95\% CI: −6.2 to 25.9).

Results from extended \textit{RAS} analysis from this trial are discussed in the “Predictive biomarkers: patient stratification” section.

The PEAK study (Panitumumab Efficacy in combination with mFOLFOX6 Against bevacizumab plus mFOLFOX6 in mCRC subjects with wild-type \textit{KRAS} tumors) was designed to address whether to include anti-EGFR vs anti-vascular endothelial growth factor (VEGF) monoclonal antibodies in the first-line treatment of patients with mCRC.\textsuperscript{37} In this Phase II, multicenter, randomized two-arm study, patients (\textit{n}=285) were randomized 1:1 to each treatment arm. The primary objective was PFS, although no formal hypothesis testing was planned. A prespecified secondary objective was to evaluate PFS and OS in \textit{RAS} wild-type patients (expanded \textit{RAS} testing included exons 2, 3, and 4 of \textit{KRAS} and \textit{NRAS}). The study was negative for its primary objective since no statistically significant difference was detected in PFS in patients with exon 2 wild-type \textit{KRAS} mCRC (PFS 10.9 vs 10.1 months, HR =0.87, \textit{P}=0.35) (Table 1). An early OS analysis (46\% deaths reported) showed, in the panitumumab arm, an improvement in OS (34.2 vs 24.3 months, HR =0.62, \textit{P}=0.009). However, a definitive separation between survival curves was not seen until approximately 15 months, suggesting that second-line therapies may have impacted these results. Indeed, patients with exon 2 wild-type \textit{KRAS} mCRC/other mutations in \textit{RAS} in the panitumumab arm were more likely to receive bevacizumab beyond progression (50\% vs 7\%) as well as cytotoxic agents (83\% vs 56\%). Alternatively, it is possible that anti-EGFR therapies may have a greater impact on OS rather than on PFS as these results are consistent with results recently reported from the Phase III FIRE-3 clinical trial.\textsuperscript{8} The analysis of secondary endpoints from the PEAK trial also provided valuable information to understand the benefit of adding anti-EGFR in different molecular subtypes. These results are discussed in the “Predictive biomarkers: patient stratification” section.

Table 1 Summary of efficacy data from PRIME\textsuperscript{36} and PEAK\textsuperscript{37} studies stratified by \textit{RAS} molecular subtypes

<table>
<thead>
<tr>
<th>Study</th>
<th>N</th>
<th>Molecular subtype</th>
<th>Panitumumab (yes vs no)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRIME</td>
<td>1,183</td>
<td>Exon 2 wt \textit{KRAS}</td>
<td>PFS 10 vs 8.6 mo, HR =0.80, \textit{P}=0.01; OS 23.8 vs 19.4 mo, HR =0.83, \textit{P}=0.03; RR 57% vs 48%, OR =1.47, \textit{P}=0.02; CRR 10% vs 8%, NS</td>
</tr>
<tr>
<td>RP3</td>
<td></td>
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<tr>
<td>RAS wt</td>
<td></td>
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</tr>
<tr>
<td>PEAK</td>
<td>285</td>
<td>Exon 2 wt \textit{KRAS}</td>
<td>PFS 10.9 vs 10.1 mo, HR =0.87, \textit{P}=0.35; OS 34.2 vs 24.3 mo, HR =0.62, \textit{P}=0.009; R0 resection 10% vs 8%; PFS 13 vs 9.5 mo, HR =0.65, \textit{P}=0.029; OS 41.3 vs 28.9, HR =0.63, \textit{P}=0.058</td>
</tr>
<tr>
<td>RP2</td>
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Note: Data from Douillard et al\textsuperscript{36} and Schwartzberg et al.\textsuperscript{37}

Abbreviations: PFS, progression-free survival; OS, overall survival; CRR, complete resection rate; NS, nonsignificant; mo, months; HR, hazard ratio; RR, response rate; OR, odds ratio; PRIME, Panitumumab Randomized trial In Combination with Chemotherapy for Metastatic Colorectal Cancer to Determine Efficacy; PEAK, Panitumumab Efficacy in Combination with mFOLFOX6 Against Bevacizumab plus mFOLFOX6 in mCRC subjects with wild-type \textit{KRAS} tumors.
Panitumumab and irinotecan schedules in first line mCRC

No data are available from Phase III randomized clinical trials testing panitumumab in combination with irinotecan schedules in the first-line setting. However, this regimen is included as one of the treatment options for KRAS/NRAS wild-type patients in the National Comprehensive Cancer Network (NCCN) and European Society for Medical Oncology guidelines. This recommendation is based on extrapolating data from study 2005-0181 in the second-line setting. In this study, the addition of panitumumab to FOLFIRI in KRAS exon 2 wild-type resulted in an improved RR (36% vs 10%, \(P<0.0001\)) and PFS (6.7 vs 4.9 months, HR = 0.82, \(P=0.023\)). A benefit in OS was not seen, possibly due to the impact of therapy beyond progression, as treatment cross-over was allowed in this study, and 90 of 119 patients (76%) in the best supportive care (BSC) group received panitumumab postprogression. 

Two Phase II studies have tested panitumumab in combination with irinotecan schedules in the first line. The first study tested panitumumab at 2.5 mg/kg weekly in combination with either IFL (irinotecan, bolus 5FU, and leucovorin) or FOLFIRI. Of all patients in the IFL arm, 58% had grade 3–4 diarrhea vs 25% in the FOLFIRI arm. No differences were seen in RRs; however, patients in the FOLFIRI arm had a trend toward increased PFS and OS.

A second single-arm, Phase II study combined panitumumab (6 mg/kg) with FOLFIRI every 2 weeks. Mutations in KRAS exon 2 were assessed in 94% of the patients. Overall, with the caveat that this was not a randomized trial, objective responses (56% vs 38%), median duration of response (13 vs 7.4 months), and \(R_{\text{F}}\) resection (8% vs 5%) were higher in the KRAS exon 2 wild-type subset.

Panitumumab plus bevacizumab in the first line

Dual inhibition of EGFR and VEGF with cetuximab and bevacizumab, in combination with irinotecan, resulted in an improved RR and PFS compared to cetuximab and bevacizumab in the BOND-2 study. This was a randomized Phase II clinical trial for patients (N=83) who had previously progressed on irinotecan. The primary objective of the study was to evaluate the safety of the combination; therefore, efficacy analysis was only exploratory. Interestingly, both arms did better than historical controls, which strongly suggest a high degree of patient selection. However, large randomized Phase III trials have failed to validate this strategy in the first-line setting. The PACCE study (Panitumumab Advanced Colon Cancer Evaluation trial) assessed dual inhibition of both EGFR and VEGF in combination with fluoropyrimidines and either oxaliplatin or irinotecan in the first-line treatment of 1,053 patients with mCRC. The primary endpoint was PFS. Regardless of KRAS status, the addition of panitumumab was detrimental in this study in regards to both PFS (10 vs 11.4 months, HR =1.27; 95% CI: 1.06–1.52) and toxicity. Grade 3 and 4 AEs were more frequent in the panitumumab arm (90% vs 77% in oxaliplatin cohort and 90% vs 63% in irinotecan cohort). Skin toxicity, diarrhea, infections, pulmonary emboli, and deep vein thrombosis were all more common in the panitumumab arm. A detrimental effect from adding cetuximab to CAPOX-bevacizumab was also observed in the Dutch Colorectal Cancer Group CAIRO-2 study.

Predictive biomarkers: patient stratification

Efforts in drug development have increasingly focused on identifying predictive biomarkers of response to enrich studies with subsets of patients most likely to benefit from targeted therapy. This strategy has, in recent years, led to expedited regulatory approval of novel targeted therapies used to treat different solid tumors.

Unfortunately, in CRC, advances in personalizing therapy have been scarce. Only RAS mutations have been identified as a negative predictive biomarker and incorporated in the clinical decision-making process.

EGFR expression

EGFR expression was initially hypothesized to be a predictive biomarker of response to EGFR monoclonal antibodies. To that end, the initial randomized trials leading to approval of these drugs in mCRC required EGFR expression by immunohistochemistry (IHC) as part of the eligibility criteria for enrollment. However, in the BOND study, a randomized clinical trial comparing cetuximab monotherapy vs cetuximab in combination with irinotecan in irinotecan refractory patients with mCRC, no correlation was found between EGFR expression in the tumor and efficacy of cetuximab. Subsequently, responses to cetuximab were seen in patients lacking EGFR expression. Therefore, it was believed that EGFR expression was not predictive of response to anti-EGFR therapies in mCRC.

KRAS exon 2 mutations

The first evidence to support the role of KRAS exon 2 mutations as a negative predictor of response in mCRC came from a randomized Phase III clinical trial testing...
panitumumab vs BSC after progression following treatment with cytotoxic agents. In this study, 427 out of 463 (92%) enrolled patients underwent KRAS exon 2 (codon 12 and 13) testing by real-time polymerase chain reaction (RT-PCR) in paraffin-embedded tumor sections. KRAS exon 2 mutations were found in 43% of patients. The primary study endpoint was PFS. In KRAS exon 2 wild-type patients, panitumumab resulted in improved PFS (12.3 vs 7.3 weeks, HR =0.45, P<0.0001). There were no differences in OS. However, crossover at the time of progression may have contributed to diluting benefit in OS. A detrimental effect from panitumumab in the KRAS mutant subset was not seen in this study. Within the population enrolled in this study, KRAS exon 2 mutations were a prognostic biomarker, as survival for this molecular subtype was shorter irrespective of treatment arm.

In the C0.17 clinical trial, 572 patients with mCRC who progressed on standard fluoropyrimidines, oxaliplatin, and irinotecan were randomized to cetuximab vs BSC. KRAS exon 2 mutations were evaluated by PCR in paraffin-embedded tumor samples from 394 (69%) of these patients. Mutations in KRAS exon 2 were found in 42% of the patients evaluated. The primary endpoint of the study was OS. In KRAS exon 2 wild-type patients, cetuximab improved OS (9.5 vs 4.8 months, HR =0.55, P<0.001) as well as PFS (3.7 vs 1.9 months, HR =0.40, P<0.001). Importantly, 7% of patients randomized to the BSC arm crossed over and received cetuximab therapy. Similar to the panitumumab trial, cetuximab had no negative impact on PFS/OS in the KRAS mutant subset. Different from that trial, in C0.17, KRAS mutations were not found to be prognostic of poor outcomes in the BSC group.

Some early data suggested that not all KRAS mutations were resistant to anti-EGFR therapy. A retrospective, pooled exploratory analysis of 579 patients treated with cetuximab in different randomized trials showed a survival benefit from cetuximab in the subset of patients with KRAS G13D mCRC (n=45, 17%). This is difficult to conciliate with the evidence that KRAS G13D clones are identified in the context of acquired resistance to anti-EGFR therapy. Indeed, a randomized Phase II study (ICECREAM) recently compared cetuximab vs cetuximab plus irinotecan in mCRC. The trial included RAS wild-type patients but allowed patients harboring G13D mutations. The study failed to show any benefit from single agent cetuximab in patients with KRAS G13D mCRC.

Therefore, NCCN guidelines do not recommend treatment with anti-EGFR therapy in patients with KRAS G13D mCRC other than in the context of a prospective clinical trial. The clinical observation that a large subset of KRAS exon 2 wild-type patients will not respond to anti-EGFR therapy has prompted investigation of additional biomarkers.

Expanded RAS analysis

Archival tissue samples from patients enrolled in the PRIME trial who had KRAS exon 2 wild-type mCRC were recently tested for additional RAS exons. Expanded RAS testing included: KRAS exon 3 (codon 61) or exon 4 (codons 117 or 146); NRAS exon 2 (codon 12 or 13), 3 (codon 61), or 4; or BRAF exon 15 (codon 600). Additional RAS mutations were found in 108 of 639 (17%) patients with KRAS exon 2 wild-type mCRC. The survival analysis according to expanded RAS results showed that the benefit from adding panitumumab was even larger in the RAS wild-type subset (Table 1). In this molecular subtype, there was a 2-month improvement in PFS (10.1 vs 7.9 months, HR =0.72, 95% CI: 0.58–0.90, P=0.004) and a 6-month improvement in OS (26 vs 20.2 months, HR =0.78, 95% CI: 0.62–0.99, P=0.04) (Figure 2). However, when panitumumab was added to FOLFOX4 in patients with a wild-type KRAS exon 2 but with a different RAS mutation, there was a nonsignificant decrease in PFS (7.3 vs 8.0 months, HR =1.28, 95% CI: 0.79–2.07, P=0.33) and OS (17.1 vs 18.3 months, HR =1.29, 95% CI: 0.79–2.10, P=0.31). These data prompted the FDA to issue a new label for panitumumab on March 11, 2015, excluding from treatment patients whose tumors had KRAS exon 2 mutations and also those who had any RAS-positive mCRC.

However, for patients with BRAF-positive mCRC there was still a trend toward increased PFS in patients treated with panitumumab in the PRIME trial (PFS – HR =0.58, 95% CI: 0.29–1.15, OS – HR =0.90). The small number of patients with BRAF-positive mCRC (n=53) may not have been sufficiently robust to evaluate the benefit of adding panitumumab in this subset in the first line. Alternatively, it is possible that adding anti-EGFR therapy in this setting is of no benefit. Indeed, cetuximab, a different EGFR mAb, was detrimental when added to fluoropyrimidines and oxaliplatin in BRAF-positive patients with mCRC treated in the first line in the COIN trial. Additionally, two recent meta-analyses showed that there was no improvement in outcomes in patients with BRAF-positive mCRC when either panitumumab or cetuximab is added to standard therapy. Consistent with this, the NCCN guidelines acknowledge that there are insufficient data to guide the use of anti-EGFR therapy in combination with chemotherapy in the first-line setting in patients with BRAF V600E mCRC. In BRAF V600E mCRC, preclinical data suggest that resistance to BRAF inhibitors is mediated through
activation of EGFR.\textsuperscript{53} For BRAF-positive patients, novel treatment strategies are urgently needed. This may include more intensive therapy or decreasing chemotherapy breaks. In this regard, a subanalysis of the TRIBE study recently showed a trend for increased survival with FOLFOXIRI compared to FOLFIRI (irinotecan with fluorouracil and folinic acid) in BRAF-positive patients, this difference was not statistically significant possibly due to the low prevalence of BRAF-positive CRC.\textsuperscript{54} Ongoing studies are evaluating BRAF inhibitors in combination with cetuximab and irinotecan or PI3K inhibitors in mCRC patients with these molecular subtypes.\textsuperscript{55,56} Early results from a dose escalation study with LGX-818 (BRAF inhibitor) plus cetuximab with or without BYL-719 (PI3K inhibitor) in patients with \textit{BRAF V600E} mCRC were presented at the AACR 2015 Annual Meeting in Philadelphia.\textsuperscript{57} In this population of patients with very poor prognoses, the RR (30\%) and median PFS (4.3 months) hold promise. In addition, a subset of these patients was able to stay on treatment beyond 44 weeks. This suggests that even within the small \textit{BRAF V600E}, subset heterogeneity is likely to influence response to therapy.

Similar to the PRIME study, the investigators in the PEAK study also retrospectively carried out additional extended \textit{RAS} testing. Results from extended \textit{RAS} testing were available in 233 out of 285 (82\%) patients from the PEAK study. Of patients with exon 2 wild-type \textit{KRAS} mCRC, 23\% had additional \textit{RAS} mutations. In \textit{RAS} wild-type patients the addition of panitumumab to FOLFOX resulted in improved PFS compared to FOLFOX plus bevacizumab (PFS 13 vs 9.5 months, HR =0.65, \textit{P}=0.029). Similarly, OS was also increased with the caveats discussed above regarding the potential impact of second-line therapy (OS 41.3 vs 28.9, HR =0.63, \textit{P}=0.058) (Figure 2). Overall RR was similar in both arms as well as rates of \textit{R}0 resection (10\% vs 8\% for panitumumab and bevacizumab, respectively). An exploratory analysis of responses from the PEAK trial limited to patients with \textit{RAS} wild-type mCRC was recently presented in abstract form.\textsuperscript{58} Responses occurred earlier (early tumor shrinkage [ETS] at 8 weeks 64\% vs 45\%, \textit{P}=0.023), were deeper (65\% interquartile range [IQR] 48–67 vs 46\%, IQR [29–62]) \textit{P}=0.0007) and lasted longer (11.4 [95\% CI 9.7–13.6] vs 8.5 [95\% CI 6.3–9.3] months, \textit{P}=0.0142) in the panitumumab arm. These results are consistent with those seen in the FIRE-3 trial. Investigators in the FIRE trial hypothesized that the depth of response may explain benefit in OS in the absence of benefit in PFS.\textsuperscript{58}

The results of expanded \textit{RAS} testing from the CRYSTAL (Cetuximab combined with Irinotecan in First-Line Therapy for Metastatic Colorectal Cancer) trial, which studied FOLFIRI with/without cetuximab as first-line therapy, showed that the incidence of expanded \textit{RAS} mutations was similar to the PRIME study.

In the CRYSTAL study, expanded \textit{RAS} testing was performed in samples available from 64\% of enrolled patients. Of all exon 2 wild-type \textit{KRAS} patients, 15\% had \textit{RAS} mutations.\textsuperscript{59} In contrast to the PRIME study, there was no negative effect seen in the CRYSTAL trial when cetuximab was added to FOLFIRI in \textit{RAS}-positive patients. The reasons

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**Figure 2** PFS and OS according to exon 2 \textit{KRAS} or \textit{RAS} status in the PRIME\textsuperscript{59} and PEAK\textsuperscript{52} trials.

**Note:** Data from Douillard et al.\textsuperscript{59} and Schwartzberg et al.\textsuperscript{52}

**Abbreviations:** PFS, progression-free survival; OS, overall survival; PRIME, Panitumumab Randomized trial In Combination with Chemotherapy for Metastatic Colorectal Cancer to Determine Efficacy; PEAK, Panitumumab Efficacy in Combination with mFOLFOX6 Against Bevacizumab plus mFOLFOX6 in mCRC subjects with wild-type \textit{KRAS} tumors.

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OncoTargets and Therapy 2016:9
Mutations in exon 20 (catalytic domain),

PI3KCA and PTEN

PI3KCA is constitutively activated in 15% of CRCs. In CRC cell lines, constitutive activation of PI3KCA leads to increased AKT1 phosphorylation. This results in decreased apoptosis and increased cell migration and invasion in vitro. Similarly, PTEN, a negative regulator of PI3K, is inactivated in 20% of CRC. PTEN loss leads to PI3K pathway activation.

A retrospective analysis of patients with mCRC treated with cetuximab across multiple centers in Europe suggested that PI3KCA mutations in exon 20 (catalytic domain), assessed by automated sequencing, and PTEN loss assessed by IHC, were associated with resistance to anti-EGFR therapy in mCRC. However, the evidence in this arena is conflicting. A different retrospective analysis from the CO.17 study (a randomized Phase III study comparing cetuximab vs BSC) arrived at different conclusions after analyzing the predictive role of PI3KCA mutations and PTEN loss. In this analysis, 407 of 572 (71%) samples were tested for PI3KCA using a nested PCR procedure; 205 patients (35%) were assessed for PTEN loss by IHC using a rabbit monoclonal antibody. Neither PI3KCA mutations nor PTEN loss were prognostic of survival in these patients. In addition, they were not predictive of benefit from cetuximab. The lack of standardized methods used to evaluate some of these biomarkers (ie, PTEN) may have contributed to these conflicting results.

MET amplifications

MET is an oncogene that encodes a tyrosine kinase receptor for hepatocyte growth factor. MET may become constitutively activated as a result of copy number variation, overexpression, or activating mutations. Genetic aberrations in MET were found in 6 (3%) out of 212 tumor specimens from CRC patients for whom both sequencing and copy number variation analyses were available according to TCGA data from cBioPortal for Cancer Genomics (www.cbioportal.org, data accessed on March 30, 2015). The most common aberrations were missense mutations found in four patients. One patient each had amplification (0.5%) and a homozygous deletion. In a recent work, MET amplifications were identified, at the time of progression to anti-EGFR therapy, in a small subset of patients with mCRC who had initially responded to EGFR inhibition. Next-generation sequencing in posttreatment biopsies collected at the time of progression identified MET amplifications in three out of seven patients. MET amplifications were then confirmed by FISH (fluorescence in situ hybridization) analysis (MET/CEP7 ratio > 3). Interestingly, in samples from patient-derived xenografts (PDXs) MET amplifications were mutually exclusive with mutations in KRAS, BRAF, NRAS, and PIK3CA and with HER2 amplification. This work suggests that MET testing could help refine the subset of patients most likely to benefit from anti-EGFR therapy. However, given the low incidence of this aberration in mCRC, large pooled analysis will be needed to confirm this hypothesis. In addition, more data are needed to define the optimal cut-off MET amplification that predicts resistance to therapy.

EGFR S492R

At the present time, the two approved anti-EGFR monoclonal antibodies are considered largely equivalent in terms of efficacy. This was studied in ASPECTT (A Study of Panitumumab Efficacy and Safety Compared to Cetuximab in Patients With KRAS Wild-Type Metastatic Colorectal Cancer), a noninferiority trial with 1,010 patients with wt-KRAS mCRC refractory to chemotherapy who were randomized to receive either panitumumab or cetuximab. OS was the same in both arms (10.4 months with panitumumab vs 10.0 months with cetuximab) and AEs were similar with slightly greater skin toxicity in the panitumumab arm although less infusion reactions (one patient vs nine in the cetuximab arm). As described above, the decreased incidence of infusion reactions is likely secondary to panitumumab being a fully human monoclonal antibody. A small Phase II trial showed no responses to panitumumab in 20 patients with mCRC who had previously progressed on cetuximab. However, acquired mutations in the extracellular domain of EGFR (including S492R, S464L, G465R, and I491M) which may predict resistance to cetuximab but not panitumumab, were recently identified. Retrospective analysis of samples from ASPECTT revealed that 16% of patients in the cetuximab arm but only 1% of those in the panitumumab arm developed EGFR S492R mutations. Those with EGFR S492R mutations in the cetuximab arm were also shown to have worse OS (11.9 vs 13.8 months, HR =1.75, 95% CI: 1.23–2.50). The role of these mutations in assisting therapeutic decisions involving the selection of the most appropriate monoclonal antibody still needs to undergo prospective validation before it can be implemented in the clinic.
p53

p53 is a tumor suppressor gene with a critical role in checkpoint activation during cell cycle progression. Mutations in p53 are present in 50% of patients with mCRC. The role of p53 as a biomarker of response to anti-EGFR therapies is still unclear. A retrospective analysis in 64 patients with chemorefractory mCRC treated with cetuximab plus chemotherapy showed a longer time to progression in those whose tumor specimens were p53 mutated vs wild-type. However, mass parallel sequencing analysis from tumor specimens collected during a Phase III trial that compared panitumumab plus BSC vs BSC alone, failed to show that p53 status was predictive of benefit from panitumumab.

HER2

Activation of HER2 signaling through HER2 amplifications or activating mutations in the kinase domain of HER2 is present in 7% of patients with advanced CRC. HER2 activation predicts resistance to EGFR inhibitor in CRC PDX. In PDX models representative of this molecular subset, dual HER2 inhibition induced tumor regression. A recent study showed promising RR (35%) in patients with HER2-positive CRC patients treated with lapatinib plus trastuzumab. HER2 was considered positive if tumor was IHC 3+ or 2+ and FISH positive (HER2:CEP17 >2).

FGFR1 amplifications and PDGFRA mutations

Whole exome sequencing of KRAS wild-type CRC PDX recently identified FGFR1 amplifications in 3% of PDX resistant to EGFR inhibition. In addition, mutations near the catalytic domain of PDGFRA were also found in 3% of tumorgrafts resistant to EGFR. Of note, single-agent pathway inhibition with BGJ398 (FGFR inhibitor) or imatinib (PDGFR inhibitor) failed to show activity in these models despite them harboring actionable aberrations. However, tumor regressions were seen when these agents were combined with cetuximab indicating that perhaps combination therapy will be needed for these molecular subsets.

Toxicity profile from first-line randomized trials

In the PRIME study, the addition of panitumumab increased the incidence of AE. This had no impact on patient-reported quality of life; however, up to 20% of the patients discontinued treatment due to AEs in the panitumumab arm. The distribution of grade 3–4 AE was similar regardless of KRAS status. The most common AE with panitumumab was acneiform rash (37% and 31% for KRAS wild-type and KRAS mutant, respectively). The incidence of skin toxicity in the control arm was less than 2%. Rash was typically located in face/upper trunk and appeared early during treatment, typically during the 1st month. In the KRAS wild-type group, patients who developed grade 2–4 skin rash had improved clinical outcomes measured by RR, PFS, and OS compared to those with grade 0–1 skin rash. Thirty percent of patients had a delayed onset of rash with similarly improved clinical outcomes as did those with early-onset rash. Data from the STEPP (Skin Toxicity Evaluation Protocol with Panitumumab) clinical trial shows that preemptive antibiotic treatment with doxycycline decreases the incidence of grade ≥2 rash secondary to panitumumab from 62% to 29%. Similarly, the addition of panitumumab increased the incidence of grade 3–4 diarrhea (18% vs 9%), hypokalemia (10% vs 5%), and fatigue (10% vs 3%).

In the PEAK study, patients in the panitumumab arm experienced AEs of any grade more frequently than those in the bevacizumab arm (91% vs 83%); however, the percentage of patients who discontinued treatment due to AEs, one-third, was similar. Again, the most common grade 3–4 AE was skin rash, 32% vs 2% in the panitumumab vs bevacizumab group. Additional grade 3–4 AEs that were increased in the panitumumab arm included hypokalemia (11% vs 5%) and hypomagnesemia (7% vs 0%).

Conclusion

The addition of panitumumab to FOLFOX in first-line treatment resulted in improved RR, PFS, and OS for patients with KRAS exon 2 wild-type mCRC. Unfortunately, the identification of predictive biomarkers of response to targeted therapy in mCRC is lagging behind compared to other malignancies. KRAS exon 2 mutations were identified as a negative predictor of response to anti-EGFR therapy in patients with mCRC nearly a decade ago. A large percentage of patients with KRAS exon 2 wild-type mCRC still derive no benefit from the addition of anti-EGFR therapies. It is clear that additional biomarkers that can be used for patient stratification are needed in this setting.

Efforts to identify new biomarkers in mCRC have included testing archival tissue from different studies with anti-EGFR therapies to search for additional mutations in 1) different exons within KRAS, 2) different members of the RAS family, 3) additional genes that may activate pathways downstream of KRAS and therefore drive resistance to therapy (HER2, PIK3CA, PTEN), and
4) genes involved in alternative signaling pathways (\textit{MET}, \textit{FGFR1}). In randomized trials with panitumumab in the first line, 17\%-23\% of \textit{KRAS} exon 2 wild-type patients harbored additional mutations within \textit{KRAS} (exon 3 and 4) or different members of the \textit{RAS} family (\textit{NRAS}). In both trials, expanded \textit{RAS} analysis was performed using dideoxy DNA Sanger sequencing (detection threshold of 5\% copies). It is important to note that expanded \textit{RAS} analysis across trials testing cetuximab has been done using different methods. Pyrosequencing was performed in samples from the FIRE-3 study. BEAMing (Bed Emulsion Amplification and Magnetics) was used in the OPUS and CRYSTAL studies (5\% sensitivity cut-off selected for the analysis). The prevalence of \textit{RAS} mutations in \textit{KRAS} exon 2 wild-type specimens from CRYSTAL, FIRE, and OPUS trials were 14.7\%, 16\%, and 31\%, respectively.

Several key questions will need to be addressed in the future. First, is a dynamic definition of expanded \textit{RAS} mutations needed or even acceptable? A recent meta-analysis showed a correlation higher than 92\% between \textit{KRAS} mutations in primary and matched metastases in CRC. However, most of those studies used detection methods with thresholds greater than 5\% per allele copies. Available technologies like BEAMing have detection thresholds as low as 0.01\%. It is plausible that if the detection threshold is lowered, additional \textit{RAS} mutations could be identified. Second, do all \textit{RAS} isoforms signify a negative predictive role? Retrospective analysis suggests this is true, at least when detection thresholds >5\% were used. However, it will be challenging to evaluate the predictive role of \textit{RAS} mutations present only in rare clones or even the individual role of some of these mutations with low prevalence. Indeed, although some early data suggested that the subgroup of patients with \textit{KRAS G13D} mutations may receive some benefit from cetuximab, the results from ICECREAM study recently showed no benefit from single agent cetuximab in patients with \textit{KRAS G13D}-positive disease. These patients should be spared from anti-EGFR therapies similar to other patients with \textit{RAS} mutations.

Third, should \textit{BRAF} be included in initial testing when data supporting the use of anti-EGFR therapy in this subset of patients in the first line are conflicting? Fourth, the role of additional biomarkers beyond \textit{RAS} will need to be better defined. For instance, the negative predictive value of \textit{PIK3CA} mutations or PTEN loss gleaned in a retrospective analysis across different European centers could not be confirmed in samples from a randomized study (C017). Finally, will the new molecular subtype classifications of CRC, largely based on gene expression analysis, allow better patient stratification and treatment selection? Results from ongoing prospective clinical trials are awaited to answer this question.

In summary, \textit{RAS} mutations have been identified as a negative predictor of response to EGFR inhibitors, including panitumumab, in patients with mCRC. A number of additional biomarkers are currently investigated for which there is conflicting evidence. As only a subset of \textit{RAS}-wild type patients benefit from anti-EGFR therapies, additional work in this arena is urgently needed to refine patient stratification.

**Disclosure**

The authors report no conflicts of interest in this work.

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