Metastatic Colorectal Cancer: Focus on Panitumumab

S. Lindsey Davis and Antonio Jimeno

University of Colorado Denver, Aurora, Colorado, USA. Email: antonio.jimeno@ucdenver.edu

Abstract: Biologic agents designed as targeted therapy are at the forefront of research in the field of colorectal cancer. Monoclonal antibodies targeting the epidermal growth factor receptor (EGFR) represent one such form of treatment, and the novel agent panitumumab is the first fully-human monoclonal antibody developed in this class. This drug is generally well-tolerated, with skin toxicities similar to those associated with other EGFR inhibitor therapies as the primary adverse effect. Efficacy has been evaluated in multiple settings, and panitumumab has demonstrated significant therapeutic effect both as monotherapy, as well as part of first- and second-line combination chemotherapy regimens. Panitumumab has also played a pivotal role in establishing the importance of biomarker science in the field of colorectal cancer and the whole of oncology. Studies demonstrating lack of therapeutic response to the agent in the setting of KRAS mutation led to the first indication of an agent based on genetic characteristics. Current and ongoing studies will continue to define panitumumab and determine its role in the treatment of colorectal cancer. This review will present background information on this therapeutic agent and explore present and future directions.

Keywords: panitumumab, EGFR, monoclonal antibody, colorectal cancer, biomarker

This article is available from http://www.la-press.com.

Rationale

As the field of oncology continues to evolve, a new focus on biologic agents designed to target specific effectors of cancer formation and spread has come to the forefront. Advances in colorectal cancer therapy have been an important part of this evolution, with multiple new treatment modalities resulting from clinical trials. Development of targeted monoclonal antibodies represents one such advancement in this area. Cetuximab, an antibody that targets the epidermal growth factor receptor (EGFR), was one of the first agents of this kind to be studied in colorectal cancer. Results of early studies demonstrated significant increase in response when added to irinotecan in irinotecan-refractory patients.[1] This and other similar data ultimately resulted in the integration of cetuximab into standard therapeutic regimens for metastatic colorectal cancer. Other monoclonal antibodies such as bevacizumab have shown similar activity in colorectal cancer,[2] and the EGFR inhibitor panitumumab has demonstrated great potential. The present role of panitumumab in the treatment of colorectal cancer as well as future directions of this novel agent will be explored in this review.

Background

Panitumumab is one in a class of monoclonal antibodies directed against the epidermal growth factor receptor. EGFR, also known as HER-1 or cErbB-1, belongs to a family of tyrosine kinase receptors with similar function as growth factor receptors. Other members of this group include HER-2, HER-3, and HER-4.[3,4] EGFR may be expressed on a variety of healthy cells, most commonly those of epithelial origin, as well as multiple types of cancer cells, where it is generally expressed in much greater numbers.[5] The receptor is activated by endogenous ligands that include epidermal growth factor (EGF) and transforming growth factor alpha (TGF-α), as well as some less active growth factors.[6] Such activation results in receptor dimerization and autophosphorylation, initiating further signaling pathway activity involving Ras, Raf, and phosphatidylinositol-3-kinase (PI3K), among others. This cascade results in cell proliferation as well as many other processes implicated in cancer formation and spread including angiogenesis, metastasis, and prevention of apoptosis.[7]

Monoclonal antibody technology has been key in the development of agents directed at this receptor. Such therapeutic antibodies have historically been generated from mice, with the first antibodies entirely murine in composition. Due to poor tolerance in humans as well as rapid clearance from the system, changes were made to decrease the murine component of these agents, first with chimeric, then humanized,[8] and ultimately fully human antibodies. With decreasing murine component, decreased immune and allergic response is expected.[9]

Cetuximab, a chimeric IgG1 immunoglobulin, was the first EGFR inhibitor approved for use in colon cancer. This approval was based on research demonstrating the clinical activity of cetuximab both alone and in combination with irinotecan after progression of colorectal cancer (CRC) on irinotecan-based regimens. Patients had significantly higher response rate (RR) and time to progression with combination therapy, though evidence of some clinically significant activity with cetuximab alone was also noted.[1] Further studies demonstrated improvement in overall survival (OS) and progression free survival (PFS) in CRC patients without response to fluoropyrimidine, irinotecan, and oxaliplatin when treated with cetuximab monotherapy compared to best supportive care (BSC).[10] More recently phase III data compared irinotecan alone to combination irinotecan/cetuximab as second-line therapy for CRC in patients previously treated with fluoropyrimidine and oxaliplatin. Results demonstrated improved PFS and RR in patients treated with combination therapy.[11]

With proven effect at this receptor site, panitumumab was subsequently developed as the first fully human antibody designed to target EGFR. Panitumumab is an IgG2 monoclonal antibody that binds EGFR with higher affinity, effectively blocking ligands EGF and TGF-α. This prevents the tyrosine phosphorylation process that initiates cell activation, and ultimately leads to apoptosis. Initial studies of panitumumab in xenograft tumor models demonstrated exactly this. Additional study demonstrated prevention of tumor formation when panitumumab was administered concomitantly with A431cells in athymic mice. Furthermore, mice with existing A431 tumor xenografts were found to have regression and complete eradication of tumor following administration of the monoclonal

antibody.[12] Follow-up investigation evaluated response to panitumumab in multiple xenograft tumor types, resulting in significant growth inhibition in those known to express relatively high levels of EGFR.[9]

Pharmacokinetics

Early studies demonstrated clearance of Panitumumab to occur by two mechanisms; through both the reticuloendothelial system, as is typical of endogenous immunoglobulins, and through EGFR itself.[13] Due to progressive EGFR saturation, decreased clearance of panitumumab initially occurs as the dose is increased, with nonlinear pharmacokinetics demonstrated at doses of 2–2.5 mg/kg. At higher doses past the point of complete receptor saturation, the reticuloendothelial system becomes the primary clearance mechanism, and pharmacokinetics become linear.[14] In a recent population pharmacokinetic model of panitumumab, median steady-state peak concentration for a standard dose of 6 mg/kg every two weeks was found to be 152 mcg/mL, with trough concentrations 34 mcg/mL. This study also noted that weight is responsible for the greatest inter-patient pharmacokinetic variability in this agent, which is decreased with weight-based dosing. Additional factors of sex, age, and cancer type are associated with some, though minimal, variability. No significant effect on pharmacokinetics was found between racial groups when comparing Japanese to non-Japanese patients.[15]

Toxicity
Phase I trials

With promising data available from animal studies, multiple phase I trials were performed to further evaluate panitumumab in terms of safety and dosing (Table 1). Dosing regimens were compared by Rowinsky et al in a trial of 88 patients with previously treated metastatic renal cell carcinoma. Patients received panitumumab at doses of 1.0, 1.5, 2.0, or 2.5 mg/kg weekly without a loading dose. Acneiform rash was found to occur at increasing rates with increasing dosages in patients receiving at least three infusions of the agent, with 100% of patients who received the 2.5 mg/kg dose developing rash. Even at high doses, human anti-human antibody (HAHA) formation was not observed, and rash formation was suggested as a possible marker of response.[14] Figlin et al evaluated panitumumab at doses from 0.1 mg/kg to 2.5 mg/kg weekly in 43 patients with various tumor types including CRC, prostate, renal, and non-small cell lung cancer (NSCLC), among others. Similar rash-response was observed at higher doses, without other documented adverse events. One patient with CRC demonstrated stable disease for four months with the 1.5 mg/kg dose.[16] A subsequent dose-escalation trial by Weiner et al provided a continuation of this data, evaluating doses of 0.01 to 5.0 mg/kg weekly, 6.0 mg/kg every two weeks, and 9.0 mg/kg every three weeks in 96 patients. After four infusions at the

Table 1. Phase I trials of panitumumab.

Study	Patient number	Tumor type	Dose (mg/kg)	Results	HAHA	Adverse Events
Rowinsky et al	88	Renal	1.0, 1.5, 2.0, or 2.5 qw	RR 3/88 SD 44/88	No	Acneiform rash in 68%, 95%, 87%, and 100% of pts by dose
Figlin et al	43	Renal, prostate NSCLC, pancreatic esophageal, CRC	0.01–2.5 qw	RR 1/43 SD 2/43	No	Acneiform rash at doses 2.0–2.5
Weiner et al	96	CRC, lung, pancreatic, renal prostate, anal, esophageal/ gastroesophageal	0.01–5.0 qw, 6.0 q2w, 9.0 q3w	RR 6/96 (5 CRC) SD 18/96	No	Dose dependent skin toxicity, plateau at >2.0 mg/kg
Stephenson et al	84	CRC, esophageal, bladder	6.0 q2w, 9.0 q3w	RR 4/84 (2/11 CRC) SD 5/11 CRC	3 pts	Adverse events in 90% of pts, most GI or skin toxicity

Abbreviations: HAHA, Human-antihuman antibody formation; qw, every week; q2w, every 2 weeks; q3w, every 3 weeks; RR, response rate; SD, Stable disease; pts, patients.

predetermined doses, no maximum tolerated dose was achieved, and the overall tolerability was not significantly different between dosing groups. Skin rash was noted to be dose dependent up to 2 mg/kg per week, and neither HAHA formation or infusion reaction was observed. Notably, five of 39 CRC patients included in the trial were found to have partial response to therapy.[17] Additional phase I testing compared 6 mg/kg dosing every two weeks as a 60 minute infusion to 6 mg/kg every two weeks as an initial 60 minute, followed by 30 minute infusions, and 9 mg/kg dosing every three weeks as a 60 minute infusion. Eighty-four patients with various previously treated solid tumors including CRC, NSCLC, and esophageal cancer were enrolled, with incidence, type and severity of adverse events similar between groups. No difference in steady-state peak concentration was noted between administration groups, and increase in drug exposure at steady state was proportional to dose. Partial response was noted in 18% and stable disease was noted in 45% of CRC patients.[18]

Skin toxicity

As suggested in above data, skin toxicity is the most common adverse event associated with panitumumab. Acneiform rash is generally described, though xerosis, nail changes, hair changes, hyperpigmentation, and telangectasias have also been reported.[19] Similar findings have been noted in EGFR inhibitors as a class. The mechanism of this toxicity remains unclear, though EGFR is expressed at high rates in various skin cells and direct receptor inhibition is thought to likely play a role.[20] In a phase II trial from Hecht et al, retrospective analysis demonstrated that patients with grade 2–4 toxicity had better PFS and OS compared with those with grade 0–1 skin toxicity.[21] Similar results were found in a Japanese trial in which patients with grade 1 skin adverse events were found to have lower response rates and shorter PFS when compared to patients with grade 2–3 skin events.[22]

Further analysis of the correlation between skin toxicity and response to panitumumab therapy was performed using data from the phase III trial comparing panitumumab to BSC. Patients in the panitumumab group demonstrating grade 2–4 skin effects were found to have a significant increase in OS compared to patients with grade 1 effects (p 0.0033). Patient-reported outcome assessments describing

more bothersome skin toxicities were also associated with longer OS. Interestingly, higher health-related quality of life was documented in patients treated with panitumumab, despite high-grade skin toxicities.[23]

Due to near-universal skin reaction in patients undergoing panitumumab therapy, studies evaluating treatment of skin toxicity have been initiated. One such study is a randomized phase II trial known as STEPP (Skin Toxicity Evaluation Protocol with Panitumumab), which compares pre-emptive versus reactive skin treatment for panitumumab-related skin toxicity. Patients receiving either panitumumab at 6 mg/kg plus FOLFIRI every two weeks or panitumumab at 9 mg/kg with irinotecan every three weeks as second-line therapy for metastatic CRC were enrolled. These patients were randomized to pretreatment for skin effects 24-hours prior to the first dose of panitumumab or reactive treatment after symptoms developed.[24] Patients in the pre-emptive treatment arm received skin moisturizer, sunscreen, 1% hydrocortisone, and doxycycline 100 mg twice daily. Results demonstrate incidence of grade 2 or greater skin toxicity in 29% of patients in the pre-emptive treatment group compared to 62% in the reactive treatment group. Patients in the pre-emptive group also reported improved quality of life as determined according to the Dermatology Life Quality Index.[25]

Other toxicities

In addition to skin toxicity, other, less common adverse effects have been noted. Diarrhea was noted in some trials of panitumumab monotherapy, but severe symptoms were rare, with only 2% of patients developing grade 3 or higher effects in a phase III trial comparing panitumumab to best supportive care.[26] This adverse effect does seem to occur at a higher rate when panitumumab is used as part of combination therapy. Diarrhea was noted to be a limiting toxicity in a trial in the IFL regimen and panitumumab were used in combination, but symptoms were tolerable and lower-grade when the agent combined with FOLFIRI instead.[27]

Hypomagnesemia has also been frequently documented in patients receiving panitumumab therapy, and similar findings have been noted with other EGFR inhibitors. This is thought to be associated with blockage of the receptor in the loop of Henle in

the kidney, where it occurs with high frequency.[28] In the phase III trial of panitumumab versus best supportive care, hypomagnesemia was found to occur in 36% of patients, but grade 3–4 symptoms occurred in only 3%.[26]

Hypersensitivity reactions are rare in panitumumab, as was originally proposed in development of this fully human monoclonal antibody. Such reactions are more commonly seen with the chimeric monoclonal antibody cetuximab, occurring in 19% of patients. Retrospective studies have demonstrated lack of hypersensitivity to panitumumab even in patients who experienced severe reaction with cetuximab therapy.[29]

Clinical Efficacy
Efficacy as monotherapy

With promising initial results from phase I trials regarding efficacy, additional studies of panitumumab as monotherapy in CRC were undertaken (Table 2), originally in patients with increased EGFR expression. Early investigation by Meropol et al evaluated patients with CRC with tumors that overexpressed EGFR by immunohistochemistry studies, and who had failed therapy with fluoropyrimidine and irinotecan or oxaliplatin. Twenty-three patients received

panitumumab at a dose of 2.5 mg/kg/week, with partial response noted in 13% and stable disease in 39%.[30] Phase II data from Berlin et al provided information regarding patients with disease progression on fluoropyrimidine, irinotecan, and oxaliplatin with EGFR staining of at least 10%.[31] One hundred eighty-two patients received panitumumab monotherapy at 6 mg/kg every two weeks with unpublished interim data at 16 weeks demonstrating partial response in 3.5% of patients according to central assessment. Additional unpublished data demonstrated a 14-week duration of response and median PFS of 7.3 weeks.

Later phase II studies also included patients with negative or low EGFR expression. Hecht et al first evaluated 148 patients with metastatic CRC who experienced treatment failure with a fluoropyrimidine and irinotecan or oxaliplatin. Tumors were evaluated for EGFR staining intensity and divided into two strata according to high (105 patients) or low (43 patients) intensity. Following treatment with panitumumab at a dose of 2.5 mg/kg/week for eight weeks, an overall RR of 9% was noted with no significant difference between strata. Stable disease was observed in 29% of patients and median PFS documented at 14 weeks with OS of 9 months, again without significant

Table 2. Trials of panitumumab as monotherapy in colorectal cancer.

Study	Patient number	Previous therapy	EGFR expression	Dose (mg/kg)	Efficacy	Median PFS/OS
Meropol et al Phase II	23	Yes	2–3 + staining in ≥10% tumor cells	2.5 qw	RR 13% SD 39%	–
Berlin et al Phase II	182	Yes	≥10% tumor cells	6 mg/kg q2w	RR 3.5%	7.3w/7.0m
Hecht et al 2007 Phase II	148	Yes	Stratum A: 2–3 + staining ≥ 10% Stratum B: 2–3 + staining ≤ 10%	2.5 qw	RR 9%* SD 29%*	14.0w/9.0m*
Hecht et al 2008 Phase II	150	Yes	Low EGFR: 1%–9% EGFR + cells (79 pts) EGFR negative: <1% EGFR + cells (71 pts)	6 mg/kg q2w	RR Low 5.1% Neg 4.2% SD Low 30% Neg 35%	Low 7.8w/8.7m Neg 8.3w/10.1m
Muro et al Phase II	52	Yes	≥1% tumor cells	6 mg/kg q2w	RR 13.5% SD 33%	8.0w/9.3m
VanCutsem et al Phase III	463	Yes	≥1% tumor cells	6 mg/kg q2w (231 pts) BSC (232 pts)	RR Pmab 10% BSC 0% SD Pmab 27% BSC 10%	Pmab 8.0w/– BSC 7.3w/–

*Data from all patients; no significant difference found between strata.
Abbreviations: pts, patients; qw, every week; q2w, every 2 weeks; RR, response rate; SD, stable disease; Pmab, Patients treated with panitumumab; BSC, patients treated with best supportive care; PFS, progression free survival; OS, overall survival; w, weeks; m, months.

difference between the two groups.[21] Additional study from Hecht et al evaluated patients with colorectal cancer refractory to standard chemotherapy with low or negative EGFR expression. One hundred fifty-eight patients were treated with panitumumab at a dose of 6 mg/kg every two weeks, with partial response noted in four patients in the low EGFR group compared to three in the EGFR negative group. Similarly, no significant difference was noted between the EGFR groups in terms of stable disease (SD), PFS, or incidence of adverse effects.[32] Finally, a phase II study of 52 Japanese patients with progression of disease on fluoropyrimidine, irinotecan and oxaliplatin evaluated effect of panitumumab at a 6 mg/kg dose every two weeks. Partial response was observed in 13.5% of patients and median PFS was noted at 8 weeks. Additional analysis demonstrated no correlation between percentage of tumor staining for EGFR and efficacy of the agent.[22]

A randomized, controlled, phase III trial further explored panitumumab as a single agent, comparing panitumumab plus BSC to BSC alone. Four hundred sixty-three patients with progression of EGFR positive metastatic CRC on fluoropyrimidine, irinotecan, and oxaliplatin were randomized. Patients in the treatment group received the agent at a 6 mg/kg dose every two weeks until disease progression or limiting toxicity. The primary end-point of progression free survival was significantly increased in the treatment group with median PFS documented at 8 weeks compared to 7.3 weeks for the BSC group. Panitumumab treatment was also associated with a higher response rate of 10% compared to 0% for BSC, and a higher stable disease rate of 27% compared to 10% in the BSC group. Overall survival was not significantly different between groups, but crossover from the BSC to panitumumab group in the setting of disease progression is thought to confound the data. Exploratory analysis excluding crossover data supports this hypothesis. These data together led to the approval of panitumumab for use both in the United States an in Europe.[26]

Additional trials further exploring panitumumab as monotherapy are ongoing. Many of these current trials evaluate only patients with an wild type form of the KRAS oncogene, the importance of which will be further discussed below. One such trial still in early stages of development is a randomized phase III study comparing panitumumab to cetuximab as monotherapy.

According to unpublished data, patients with metastatic wild-type (WT) KRAS colorectal cancer who have progressed on irinotecan and oxaliplatin will be recruited to the ASPECCT/20080763 trial with a goal enrollment of 1000. These patients will be randomized to cetuximab or panitumumab therapy with overall survival as the primary end point. Further similar study has been approved to evaluate panitumumab therapy in patients with disease progression on cetuximab. The phase II trial 20070602 has been designed to evaluate CRC patients with wild-type KRAS tumors who experienced treatment failure following 4 or more weeks of cetuximab therapy per unpublished data. These patients will be treated with panitumumab monotherapy every two weeks, with response rate monitored as the primary outcome measure.

Efficacy in combination with chemotherapy

With positive results in trials of panitumumab used as monotherapy in CRC patients with treatment failure on standard chemotherapy regimens, additional studies have been performed to evaluate the monoclonal antibody as part of combination chemotherapy (Table 3). Berlin, et al performed a phase II trial to this end, assessing panitumumab in combination with irinotecan, 5-fluorouracil, and leucovorin in the first-line setting. Nineteen patients were enrolled in part one of the study, in which bolus 5-FU (IFL) was used in combination with the monoclonal antibody. Due to severe toxicity with grade three diarrhea in 58% of patients, the additional 24 patients received infusional 5-FU (FOLFIRI) in part two of the trial. Response rates were similar between the two groups with 46% in part one and 42% in part two, but PFS and OS was greater in the panitumumab/FOLFIRI group. Furthermore, this regimen was better tolerated with a decreased rate of high-grade diarrhea as noted above.[27]

Panitumumab as part of first-line combination therapy for metastatic CRC was further explored in the Panitumumab Advanced Colorectal Cancer Evaluation (PACCE) study. This trial compared bevacizumab and oxaliplatin-based or bevacizumab and irinotecan-based regimens with and without panitumumab at a dose of 6 mg/kg every two weeks. Interim data demonstrated a shorter PFS (8.8 vs. 10.5 months) and increased high-grade toxicity

Table 3. Trials of panitumumab in combination therapy in colorectal cancer.

Study	Phase	Patient number	Line of therapy	Regimen	RR	Median PFS	Median OS
Berlin et al	II	43	First	Pmab 2.5 mg/kg/w + IFL	46%	5.6m	17m
				Pmab 2.5 mg/kg/w + FOLFIRI	42%	10.9m	22.5m
Hecht et al	III	1050	First	Pmab 6 mg/kg q2w +	46%	10.0m	19.4m
				Ox-CT + Bev			
PACCE				Ox-CT + Bev	48%	11.4m	24.5m
20040249				Pmab 6 mg/kg q2w + Iri-CT +	43%	10.1m	20.7m
				Bev Iri-CT + Bev	40%	11.7m	20.5m
Douillard et al	III	1,183	First	Pmab 6 mg/kg + FOLFOX4	55%	9.6m	NA
PRIME				q2w (wt) FOLFOX4 q2w (wt)	48%	8m	
20050203				Pmab 6 mg/kg + FOLFOX4q2w		7.3m	
				(mut) FOLFOX4 q2w (mut)		8.8m	
PEAK	II	280	First	Pmab + FOLFOX6	NA	NA	NA
20070509		planned		Bev + FOLFOX6			
Greil et al	II	154	First	Pmab 6 mg/kg + FOLFIRI q2w	NA	NA	NA
20060314				(Single arm)			
Peeters et al	III	1,186	Second	Pmab 6 mg/kg + FOLFIRI	NA	5.9m	14.5m
20050181				q2w (wt)			
				FOLFIRI q2w (wt)		3.9m	12.5m
				Pmab 6 mg/kg + FOLFIRI		5.0m	11.8m
				q2w (mut)			
				FOLFIRI q2w (mut)		4.9m	11.1m
SPIRITT	II	277	Second	Pmab 6 mg/kg + FOLFIRI	NA	NA	NA
20060141				q2w Bev + FOLFIRI q2w			
VOXEL	II	174	Second	Pmab 9 mg/kg q3w +	NA	NA	NA
AIOKRK0107				XELOX XELOX			
TTD-06-04	II	80	Second	Pmab 9 mg/kg q3w +	NA	NA	NA
				Irinotecan (Single arm)			

Abbreviations: pmab, panitumumab; w, week; q2w, every 2 weeks; q3w, every 3 weeks; Ox-CT, fluorouracil, leucovorin, oxaliplatin-based chemotherapy; Iri-CT, fluorouracil, leucovorin, and irinotecan-based chemotherapy; Bev, bevacizumab; wt, wild type KRAS; mut, mutated KRAS; RR, response rate; PFS, progression free survival; OS, overall survival; NA, not available; m, months.

in the panitumumab/oxaliplatin/bevacizumab group, with grade 4 adverse events including diarrhea, infection, and pulmonary embolism. Due to these results, the PACCE study was discontinued. Additional results from each stratum were later reported in final analysis, with 823 patients in the oxaliplatin-based group demonstrating a median PFS of 10 months with panitumumab versus 11.4 months with standard therapy but a similar RR of 46% versus 48%. Increased adverse events were also noted in the panitumumab group, and were similar to those noted in the interim analysis. Further evaluation of results from the 230 patients in the irinotecan-based group demonstrated median PFS of 10.1 months in the panitumumab group compared to 11.7 months in the standard therapy group. No significant difference was noted in RR, with a rate of 43% associated with panitumumab therapy compared to 40% with standard therapy. However, intolerable adverse effects were again increased in the panitumumab group. With these results, use of panitumumab with bevacizumab and combination chemotherapy is not recommended.[33]

Trials investigating other combination chemotherapy for first-line treatment of metastatic CRC have also been performed. The combination of FOLFOX

chemotherapy with panitumumab for first-line was investigated in the randomized phase III study PRIME/20050203.[34] A total of 1,183 patients were randomized to FOLFOX4 with panitumumab at 6 mg/kg dose every two weeks versus FOLFOX4 alone. Patients in the FOLFOX4/panitumumab group with wild-type KRAS demonstrated median PFS of 9.6 months and RR of 55% compared to PFS of 8 months and RR 48% in patients with unmutated KRAS treated with FOLFOX4 alone.[35] Also in progress is a phase II trial comparing panitumumab and FOLFOX6 combination therapy to bevacizumab and FOLFOX6 as first-line therapy in patients with unresectable wild-type KRAS metastatic CRC. According to unpublished data, progression-free survival is the primary outcome measure in this trial known as PEAK. Panitumumab plus FOLFIRI as a first-line therapy is also being evaluated. The single arm multicentre phase II study 20060314 is underway to evaluate response to panitumumab and FOLFIRI combination therapy in previously untreated patients with metastatic CRC. At the time of interim analysis, 154 patients have been enrolled, with overall good tolerance of the regimen.[36]

Studies evaluating panitumumab as part of combination therapy in the second-line setting have also been performed. Study 20050181 is a randomized phase III trial comparing FOLFIRI plus panitumumab at a dose of 6 mg/kg every two weeks to FOLFIRI alone as second-line therapy.[37] A total of 1,186 patients with metastatic CRC who experienced disease progression on a 5-FU-based regimen were enrolled. Patients with wild-type KRAS in the panitumumab group were found to have longer PFS of 5.9 months compared to 3.9 months in the monotherapy group. Similarly, OS was significantly increased in the FOLFIRI/panitumumab group with 14.5 versus 12.5 months documented in wild-type KRAS patients. No significant difference in PFS or OS was noted in patients with KRAS mutations.[35] According to unpublished data, a phase II trial comparing FOLFIRI with panitumumab to FOLFIRI with bevacizumab as second-line treatment for patients with wild-type KRAS metastatic CRC and disease progression on oxaliplatin-based chemotherapy with bevacizumab is also underway. Progression free survival is the primary outcome measure in this trial known as SPIRITT/20060141.

The combination of panitumumab and XELOX in the second-line setting is also being investigated in an unpublished phase II study known as VOXEL. Patients with WT KRAS metastatic CRC who failed first-line therapy with fluoropyrimidine and irinotecan are being randomized to panitumumab plus oxaliplatin and capecitabine or oxaliplatin and capecitabine alone to evaluate PFS according to early reports.

Use of panitumumab as part of combination chemotherapy in the third-line setting is presently being studied in the phase II trial CDR0000593012. Patients with WT KRAS metastatic CRC who had progression of disease on a combination regimens including FOLFOX, FOLFIRI, CAPIRI, and XELOX with or without bevacizumab and irinotecan will be treated with panitumumab/irinotecan combination therapy, with RR as the primary outcome measure per unpublished reports.

Use in locally advanced colorectal cancer

Additional trials are evaluating panitumumab in the setting of neoadjuvant therapy for locally advanced CRC without metastases. Patients with stage T4 or poor prognosis colon cancer without associated metastasis are being recruited to the phase III study CDR0000590089 to compare combination therapy with fluorouracil, oxaliplatin, and panitumumab to fluorouracil and oxaliplatin alone prior to surgical resection. Per unpublished data, incidence of recurrent or persistent disease and change in pathologic stage with therapy will be compared between the groups. The unpublished phase II trial CDR0000629101 is also investigating use of panitumumab in the neoadjuvant setting for locally advanced rectal cancer. Patients with stage T3-4 N1-2 M0 adenocarcinoma of the rectum will be randomized to panitumumab plus capecitabine and radiation therapy or capecitabine and radiation alone prior to resection. Pathological response will be measured on tumor specimens according to Dworak grade. A similar phase II trial CDR0000652936 is underway to evaluate neoadjuvant radiation, capecitabine, and panitumumab with and without irinotecan in patients with localized rectal cancer. Pathologic tumor response and occurrence of high-grade toxicities are of greatest concern in this unpublished work.

Use as neoadjuvant therapy in metastatic cancer

The role of panitumumab as a neoadjuvant therapy is also being studied in patients with metastatic colorectal cancer. A retrospective study of twelve patients treated with FOLFOX and either panitumumab or cetuximab prior to resection of liver metastases was performed in order to further investigate response to and tolerability of these regimens, as well as their effect on resectability rates. A partial response was noted in 10 of 12 patients (83%), and stable disease in the remaining 2 patients. In addition, largest measurable liver lesions were noted to have a mean decrease in size of 46%. It was thus concluded that pre-operative combination therapy with FOLFOX and an EGFR inhibitor is associated with a high response rate and may increase resectability rates.[38] Evaluation of combination therapy with panitumumab plus FOFOX 4 and panitumumab plus FOLFIRI is currently underway in patients with colorectal cancer and unresectable liver metastases. According to unpublished data, this phase II trial known as PLANET is designed to study the efficacy and safety of each of these combinations, with a focus on the conversion of unresectable liver metastases to resectable disease.

Biomarkers
EGFR expression

Despite promising data regarding clinical efficacy, overall response rates were noted to be quite variable in patients treated with panitumumab and other EGFR inhibitors. As such, additional factors have been explored on the molecular level as possible contributors to these irregularities. Initially, EGFR expression was proposed as a primary determinant of response, based on the concept that higher receptor number would likely result in greater EGFR inhibitor activity. However, as noted above, multiple panitumumab trials have investigated response in terms of EGFR receptor expression through staining intensity, and have consistently demonstrated no difference.[21,22,32] Studies of EGFR inhibitor cetuximab have provided similar results. In the randomized trial comparing cetuximab plus irinotecan to cetuximab alone, higher levels of EGFR expression determined by either percentage of EGFR-positive cells or maximal staining intensity were not found to be associated with increased clinical response.[1] Furthermore, in a retrospective analysis of EGFR-negative patients in a non-study setting, 4 of 16 patients treated with cetuximab as monotherapy or as part of combination treatment were found to have an objective response. This 25% response rate is similar to that of EGFR positive patients treated with cetuximab.[39] These results raised questions regarding the validity of EGFR testing according to these methods in predicting response to EGFR inhibitor therapy, and such evaluation has now fallen out of favor.

Other measures of EGFR expression have subsequently been explored, with EGFR gene copy number (GCN) representing one such technology. In early studies, EGFR GCN was evaluated in 31 patients with metastatic colorectal cancer treated with cetuximab or panitumumab. Increased GCN was noted in 8 of 9 patients with OR, and only 1 of 21 non-responders were found to have increased GCN.[40] Additional exploratory data based on information from the phase III panitumumab versus BSC trial further addressed this issue. Tumor samples from 58 patients receiving panitumumab and 34 patients receiving BSC were evaluated by FISH for GCN and percentage of chromosome 7 polysomia. Shorter PFS and OS was noted in patients with mean GCN less than 2.5 and chromosome 7 polysomy in less than 40% of cells.[41]

More recently, EGFR GCN has been evaluated in the setting of cetuximab therapy. Both FISH and CISH technologies were used to determine GCN in a trial of 44 patients with metastatic colorectal cancer undergoing second- or third-line therapy with irinotecan and cetuximab. Partial remission was observed in 60% patients with GCN at or above 2.6 by FISH. Thirty-six percent of patients with EGFR GCN greater than or equal to 2.12 by CISH were found to have partial remission. Overall, median time to progression was increased at 7.7 months in these groups compared to 6.4 months in those with low GCN by either method.[42]

KRAS

With EGFR receptor number by staining found to be an inconsistent predictor of response to panitumumab

and other EGFR inhibitors, evaluation of other possible effectors of response was pursued. KRAS, an oncogene that encodes proteins key to normal cellular proliferation in the EGFR pathway, was at the forefront of this search. Oncogenic mutations in KRAS are known to result in the formation of constitutively functioning proteins that lead to continuous activation of downstream pathways, an event commonly seen in tumor cells.[43] Furthermore, mutations in KRAS are commonly seen in colorectal cancer, occurring at rates up to 50%, and in past studies have been associated with poor prognosis.[44]

The importance of KRAS mutation in the setting of EGFR inhibitor therapy was further explored in subgroup analysis of the phase III trial comparing panitumumab monotherapy to BSC. Biomarker analyses were performed on tumor samples to identify mutant versus wild-type (WT) KRAS with PFS compared between groups. Patients with KRAS mutations demonstrated no significant change in PFS with panitumumab therapy compared to BSC, while patients with WT KRAS were found to have a median PFS of 12.3 weeks with panitumumab therapy compared to 7.3 weeks with BSC, representing a statistically significant difference in treatment effect ($p < 0.0001$). The response in patients with WT KRAS was not universal, however, indicating additional factors play a role in treatment resistance.[45] Further data from the Hecht et al trial comparing low to complete absence of EGFR expression also included evaluation of KRAS status. Of the 8 patients with OR, 7 had WT KRAS evaluated by PCR.[32] A recent single arm study of panitumumab at 6 mg/kg plus FOLFIRI every 2 weeks was performed by Cohn et al in 109 patients with metastatic CRC and disease progression on oxaliplatin-based chemotherapy plus bevacizumab first line.[46] Patients were evaluated according to KRAS status, with OR of 23% in WT KRAS patients versus 16% in those with mutated KRAS. Median PFS was 26 weeks in wild-type versus 19 weeks in mutated patients, with median OS 50 versus 31 weeks.[35] Additional studies have reinforced these findings with similar results, as noted above.[35] A systematic review is currently ongoing to further evaluate randomized trials addressing the role of KRAS mutation in response to EGFR inhibitor therapy. Preliminary data of 15 studies in which patients received either panitumumab or cetuximab therapy indicates an increase in PFS in patients with WT KRAS compared to those with mutant form.[47]

The overwhelming results of these and similar studies were largely responsible for the specific approval guidelines of the European Medicines Agency (EMEA) to provide panitumumab only for patients with wild-type KRAS. Based on this same concept, the FDA recently approved labeling changes on panitumumab and cetuximab recommending use only in patients without KRAS mutations. Such decisions mark an important transition in the field of CRC that places more emphasis on biomarker science.

There are some logistical concerns, however, mostly involving KRAS mutation testing. New research has begun to address this issue, evaluating both sample processing and mutation detection. In terms of processing, results of unpublished data showed core sampling to be more favorable than section preparation, with advantages of greater efficiency, lower cost, fewer false positives, and a 10%–20% increase in the detection of mutations. The same research also addressed the process of mutation detection, comparing traditional direct sequencing to high resolution melting (HRM) analysis and amplification refractory mutation system (ARMS) testing. Though the generally accepted gold standard, direct sequencing is a detailed process requiring interpretation by experienced personnel, and is not generally designed for clinical use. Comparison of this to newer technologies revealed a lower mutation detection frequency when direct sequencing was used. Though the newer technologies demonstrated similar rates of mutation detection, HRM was associated with a higher false positive rate, making ARMS the most sensitive and specific test evaluated (personal communication, A. Jimeno November 2009).

BRAF

Even patients with WT KRAS have not shown complete response to EGFR inhibitor therapy, and as such, other parts of the EGFR signaling pathway have been evaluated as possible contributing factors. One potential contributor is BRAF, a serine-threonine kinase that acts as a downstream effector of KRAS. Mutation of this gene has been proposed to result in pathway activation similar to that of KRAS. Retrospective analysis was performed on 113 patients with metastatic CRC previously treated with cetuximab or panitumumab to further investigate the effect of BRAF mutation. The BRAF V600E mutation was noted in 11 of 79 patients with WT KRAS; no patient

in this group demonstrated response to therapy, and a shorter PFS and OS were noted when compared to patients with WT BRAF. Additional cellular models of CRC in this trial demonstrated decreased response to cetuximab or panitumumab in cells with the BRAF V600E mutation. When RAF inhibitor sorafenib was added to EGFR inhibitor therapy, improved response in BRAF mutated cells was noted.[48] With these findings, further trials have been initiated to evaluate combined therapy with cetuximab and sorafenib.[49]

Additional evaluation of BRAF mutation has been performed with similar results. Retrospective analysis of tumors demonstrating BRAF mutations in the setting of WT KRAS was performed in a trial evaluating patients with irinotecan-refractory metastatic CRC subsequently treated with cetuximab/irinotecan therapy. Results demonstrated no response to therapy in 13 patients with BRAF mutations, as well as a trend to shorter PFS.[50] Another investigation was performed as a retrospective analysis in a trial comparing combination chemotherapy, bevacizumab, and cetuximab for the treatment of metastatic CRC to chemotherapy and bevacizumab alone. In this study of 516 patients, 8.7% were found to have BRAF V600E mutations. These patients demonstrated shorter PFS and OS, regardless of treatment group, leading the authors to suggest that BRAF represents a poor prognostic factor in general, with poor response even to treatments not directed at EGFR.[51]

PIK3CA

The lipid kinase PIK3CA is another EGFR pathway effector that has been considered to play a role in EGFR inhibitor response. Specific mutations of the PIK3CA gene are noted to be oncogenic in cellular models, and are thought to be associated with metastasis and decreased apoptosis.[52] Sartore-Bianchi et al further explored the relevance of PIK3CA mutation through retrospective analysis of 110 patients with metastatic CRC previously treated with panitumumab or cetuximab therapy. Of the 15 patients with PIK3CA mutations, none demonstrated objective tumor response to EGFR inhibitor therapy, and an overall trend of decreased PFS was observed.[53] Study of PIK3CA by Prenen, et al, demonstrated different results. In this retrospective analysis, tumors from 200 patients with metastatic CRC treated with cetuximab were evaluated for PIK3CA mutations. Twenty-three

patients were found to have such a mutation, with objective response to cetuximab reported in five.[54]

PTEN

In the same PI3K pathway, tumor suppressor PTEN works to control cellular proliferation. Loss of PTEN expression has also been suggested as a contributing factor to EGFR inhibitor resistance. A retrospective analysis of 27 patients previously treated with cetuximab evaluated this possibility. No objective benefit of cetuximab therapy was observed in the eleven patients found to have loss of PTEN protein expression, while 62.5% of patients with normal expression had an objective response.[55] Sartore-Bianchi et al also evaluated the effect of PTEN protein status in a retrospective analysis of 110 patients with metastatic CRC treated with EGFR inhibitor therapy. Loss of PTEN was associated with shorter PFS, but was not statistically significant ($p = 0.0681$); however, a significant decrease in OS was noted with this aberration ($p = 0.0048$).[53]

Conclusions

The data presented has shown panitumumab to be an important new therapy in the field of colorectal cancer. The first fully-human monoclonal antibody approved for use in CRC, it is generally well-tolerated, with skin toxicity the primary adverse event. It has demonstrated significant activity in a variety of settings, in some cases providing a therapeutic option to patients who otherwise would not have one. Application of the monoclonal antibody to further combinations of therapy and clinical settings continue to be explored, with hopes of maximizing its benefit. Even more, panitumumab has played a pivotal role in bringing biomarker science to the forefront of colorectal cancer therapy as well as the field of oncology.

Further clinical trials are necessary to address the many aspects of panitumumab that remain unclear. Though KRAS mutation status has helped guide decisions regarding administration of this new therapy, there is still much to learn about other genetic factors that are also likely associated with response to therapy. Prospective studies of BRAF and others should be pursued to this end. Farther ahead, the logistics of bringing genetic and molecular testing and technique to the clinical setting will become

increasingly important. With these and other ideas to explore, it will be exciting to see the ultimate role that panitumumab will play in furthering the field of colorectal cancer and the whole of oncology.

Disclosures

This manuscript has been read and approved by all authors. This paper is unique and is not under consideration by any other publication and has not been published elsewhere. The authors report no conflicts of interest.

References

1. Cunningham D, Humblet Y, Siena S, et al. Cetuximab monotherapy and cetuximab plus irinotecan in irinotecan-refractory metastatic colorectal cancer. *N Engl J Med*. 2004;351(4):337–45.
2. Hurwitz H, Fehrenbacher L, Novotny W, et al. Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. *N Engl J Med*. 2004;350(23):2335–42.
3. Carpenter G. Receptors for epidermal growth factor and other polypeptide mitogens. *Annu Rev Biochem*. 1987;56:881–914.
4. Carpenter G, Cohen S. Epidermal growth factor. *J Biol Chem*. 1990;265(14):7709–12.
5. Thompson D, Gill G. The EGF receptor: structure, regulation and potential role in malignancy. *Cancer Surv*. 1985;4(4):767–88.
6. Goustin A, Leof E, Shipley G, Moses H. Growth factors and cancer. *Cancer Res*. 1986;46(3):1015–29.
7. Wells A. EGF receptor. *Int J Biochem Cell Biol*. 1999;31(6):637–43.
8. Owens R, Young R. The genetic engineering of monoclonal antibodies. *J Immunol Methods*. 1994;168(2):149–65.
9. Yang XD, Jia XC, Corvalan JR, Wang P, Davis CG. Development of ABX-EGF, a fully human anti-EGF receptor monoclonal antibody, for cancer therapy. *Crit Rev Oncol Hematol*. 2001;38(1):17–23.
10. Jonker D, O'Callaghan C, Karapetis C, et al. Cetuximab for the treatment of colorectal cancer. *N Engl J Med*. 2007;357(20):2040–8.
11. Sobrero A, Maurel J, Fehrenbacher L, et al. EPIC: phase III trial of cetuximab plus irinotecan after fluoropyrimidine and oxaliplatin failure in patients with metastatic colorectal cancer. *J Clin Oncol*. 2008;26(14):2311–9.
12. Yang X, Jia X, Corvalan J, Wang P, Davis C, Jakobovits A. Eradication of established tumors by a fully human monoclonal antibody to the epidermal growth factor receptor without concomitant chemotherapy. *Cancer Res*. 1999;59(6):1236–43.
13. Roskos L, Arends R, Lohner M, et al. Optimal dosing of Panitumumab (ABX-EGF) in cancer patients. In: *18th UICC International Cancer Congress*; 2002.
14. Rowinsky EK, Schwartz GH, Gollob JA, et al. Safety, pharmacokinetics, and activity of ABX-EGF, a fully human anti-epidermal growth factor receptor monoclonal antibody in patients with metastatic renal cell cancer. *J Clin Oncol*. 2004;22(15):3003–15.
15. Ma P, Yang BB, Wang YM, et al. Population pharmacokinetic analysis of panitumumab in patients with advanced solid tumors. *J Clin Pharmacol*. 2009;49(10):1142–56.
16. Figlin R, Belldegrun S, Crawford J, et al. ABX-EGF, a fully human anti-epidermal growth factor receptor monoclonal antibody in patients with advanced cancer: phase 1 clinical results. In: *ASCO Annual Meeting*; 2002.
17. Weiner L, Belldegrun E, Rowinsky E, et al. Updated results from a dose and schedule study of Panitumumab (ABX-EGF) monotherapy, in patients with advanced solid malignancies. In: *ASCO Annual Meeting*; 2005.
18. Stephenson JJ, Gregory C, Burris H, et al. An open-label clinical trial evaluating safety and pharmacokinetics of two dosing schedules of panitumumab in patients with solid tumors. *Clin Colorectal Cancer*. 2009;8(1):29–37.
19. Segaert S, Van Cutsem E. Clinical signs, pathophysiology and management of skin toxicity during therapy with epidermal growth factor receptor inhibitors. *Ann Oncol*. 2005;16(9):1425–33.
20. Robert C, Soria J, Spatz A, et al. Cutaneous side-effects of kinase inhibitors and blocking antibodies. *Lancet Oncol*. 2005;6(7):491–500.
21. Hecht JR, Patnaik A, Berlin J, et al. Panitumumab monotherapy in patients with previously treated metastatic colorectal cancer. *Cancer*. 2007;110(5):980–8.
22. Muro K, Yoshino T, Doi T, et al. A phase 2 clinical trial of panitumumab monotherapy in Japanese patients with metastatic colorectal cancer. *Jpn J Clin Oncol*. 2009;39(5):321–6.
23. Peeters M, Siena S, Van Cutsem E, et al. Association of progression-free survival, overall survival, and patient-reported outcomes by skin toxicity and KRAS status in patients receiving panitumumab monotherapy. *Cancer*. 2009;115(7):1544–54.
24. Mitchell E, LaCouture M, Shearer H, et al. A phase II, open-label, randomized clinical trial of skin toxicity evaluation (STEPP) in metastatic colorectal cancer patients receiving 2nd line FOLFIRI or irrinotecan-only chemotherapy with panitumumab: Early analysis. In: *ASCO Gastrointestinal Cancers Symposium*; 2008.
25. Lacouture M, Mitchell E, Shearer H, et al. Impact of pre-emptive skin toxicity and treatment on panitumumab-related skin toxicities and quality of life in patients with metastatic colorectal cancer: Results from STEPP. In: *ASCO 2009 Gastrointestinal Cancers Symposium*; 2009.
26. Van Cutsem E, Peeters M, Siena S, et al. Open-label phase III trial of panitumumab plus best supportive care compared with best supportive care alone in patients with chemotherapy-refractory metastatic colorectal cancer. *J Clin Oncol*. 2007;25(13):1658–64.
27. Berlin J, Posey J, Tchekmedyian S, et al. Panitumumab with irinotecan/leucovorin/5-fluorouracil for first-line treatment of metastatic colorectal cancer. *Clin Colorectal Cancer*. 2007;6(6):427–32.
28. Schrag D, Chung K, Flombaum C, Saltz L. Cetuximab therapy and symptomatic hypomagnesemia. *J Natl Cancer Inst*. 2005;97(16):1221–4.
29. Power DG, Shah MA, Asmis TR, Garcia JJ, Kemeny NE. Safety and efficacy of panitumumab following cetuximab: retrospective review of the Memorial Sloan-Kettering experience. *Invest New Drugs*. 2009.
30. Meropol N, Berlin J, Hecht J, et al. Multicenter study of ABX-EGF monotherapy in patients with metastatic colorectal cancer. In: *2003 ASCO Annual Meeting*; 2003.
31. Berlin J, Neubauer M, Swanson P, et al. Panitumumab antitumor activity in patients with metastatic colorectal cancer expressing >10% epidermal growth factor receptor. In: *2006 ASCO Annual Meeting*; 2006.
32. Hecht J, Mitchell E, Baranda J, et al. Panitumumab efficacy in patients with metastatic colorectal cancer with low or undetectable levels of epidermal growth factor receptor: Final efficacy and KRAS analysis. In: *ASCO 2008 Gastrointestinal Cancers Symposium*; 2008.
33. Hecht JR, Mitchell E, Chidiac T, et al. A randomized phase IIIB trial of chemotherapy, bevacizumab, and panitumumab compared with chemotherapy and bevacizumab alone for metastatic colorectal cancer. *J Clin Oncol*. 2009;27(5):672–80.
34. Douillard J, Siena S, Cassidy J, et al. Phase III study (PRIME/20050203) of panitumumab with FOLFOX4 compared to FOLFOX4 alone in patients with previously untreated metastatic colorectal cancer: Preliminary safety data. In: *ASCO 2008 Gastrointestinal Cancers Symposium*; 2008.
35. Colorectal cancer highlights from the 2009 joint ECCO/ESMO multi-disciplinary congress. *Clinical Advances in Hematology and Oncology*. 2009;7(10).
36. Greil R, Letocha H, Gamelin E, et al. Updated analysis of a phase II study (20060314) of panitumumab with FOLFIRI as first-line treatment of patients with metastatic colorectal cancer. In: *2009 ASCO Annual Meeting*; 2009.
37. Peeters M, Wilson G, Hotko Y, et al. Phase III study (20050181) of panitumumab with FOLFIRI compared to FOLFIRI alone as second-line treatment in patients with metastatic colorectal cancer: Preliminary safety results. In: *ASCO 2008 Gastrointestinal Cancers Symposium*; 2008.
38. Tan B, Zubal B, Hawkins W, et al. Preoperative FOLFOX plus cetuximab or panitumumab therapy for patients with potentially resectable hepatic colorectal metastases. In: *ASCO 2009 Gastrointestinal Cancers Symposium*; 2009.

39. Chung K, Shia J, Kemeny N, et al. Cetuximab shows activity in colorectal cancer patients with tumors that do not express the epidermal growth factor receptor by immunohistochemistry. *J Clin Oncol.* 2005;23(9):1803–10.

40. Moroni M, Veronese S, Benvenuti S, et al. Gene copy number for epidermal growth factor receptor (EGFR) and clinical response to antiEGFR treatment in colorectal cancer: a cohort study. *Lancet Oncol.* 2005;6(5):279–86.

41. Sartore-Bianchi A, Moroni M, Veronese S, et al. Epidermal growth factor receptor gene copy number and clinical outcome of metastatic colorectal cancer treated with panitumumab. *J Clin Oncol.* 2007;25(22):3238–45.

42. Scartozzi M, Bearzi I, Mandolesi A, et al. Epidermal Growth Factor Receptor (EGFR) gene copy number (GCN) correlates with clinical activity of irinotecan-cetuximab in K-RAS wild-type colorectal cancer: a fluorescence in situ (FISH) and chromogenic in situ hybridization (CISH) analysis. *BMC Cancer.* 2009;9:303.

43. Downward J. Targeting RAS signalling pathways in cancer therapy. *Nat Rev Cancer.* 2003;3(1):11–22.

44. Andreyev H, Norman A, Cunningham D, Oates J, Clarke P. Kirsten ras mutations in patients with colorectal cancer: the multicenter "RASCAL" study. *J Natl Cancer Inst.* 1998;90(9):675–84.

45. Amado RG, Wolf M, Peeters M, et al. Wild-type KRAS is required for panitumumab efficacy in patients with metastatic colorectal cancer. *J Clin Oncol.* 2008;26(10):1626–34.

46. Cohn A, Smith D, Neubauer M, et al. Effect of tumor KRAS mutation status on second-line treatment with panitumumab and FOLFIRI: Interim results from the Pmab Regimen Evaluation in Colorectal Cancer to Estimate Primary Response to Treatment (PRECEPT) study. In: *ASCO 2009 Gastrointestinal Cancers Symposium*; 2009.

47. Vale C, Tierney J, Meade A, et al. Impact of K-ras status on the effects of EGFR-targeted monoclonal antibody therapy in advanced colorectal cancer: A systematic review and meta-analysis of randomized controlled trials. In: *2009 ASCO Annual Meeting*; 2009.

48. Di Nicolantonio F, Martini M, Molinari F, et al. Wild-type BRAF is required for response to panitumumab or cetuximab in metastatic colorectal cancer. *J Clin Oncol.* 2008;26(35):5705–12.

49. Selected clinical trials in colorectal cancer. *Clinical Colorectal Cancer.* 2007;6(7):539.

50. Loupakis F, Ruzzo A, Cremolini C, et al. KRAS codon 61, 146 and BRAF mutations predict resistance to cetuximab plus irinotecan in KRAS codon 12 and 13 wild-type metastatic colorectal cancer. *Br J Cancer.* 2009;101(4):715–21.

51. Tol J, Nagtegaal I, Punt C. BRAF mutation in metastatic colorectal cancer. *N Engl J Med.* 2009;361(1):98–9.

52. Samuels Y, Diaz LJ, Schmidt-Kittler O, et al. Mutant PIK3CA promotes cell growth and invasion of human cancer cells. *Cancer Cell.* 2005;7(6):561–73.

53. Sartore-Bianchi A, Martini M, Molinari F, et al. PIK3CA mutations in colorectal cancer are associated with clinical resistance to EGFR-targeted monoclonal antibodies. *Cancer Res.* 2009;69(5):1851–7.

54. Prenen H, De Schutter J, Jacobs B, et al. PIK3CA mutations are not a major determinant of resistance to the epidermal growth factor receptor inhibitor cetuximab in metastatic colorectal cancer. *Clin Cancer Res.* 2009;15(9):3184–8.

55. Frattini M, Saletti P, Romagnani E, et al. PTEN loss of expression predicts cetuximab efficacy in metastatic colorectal cancer patients. *Br J Cancer.* 2007;97(8):1139–45.