Biomarkers Predicting Clinical Outcome of Epidermal Growth Factor Receptor–Targeted Therapy in Metastatic Colorectal Cancer

Salvatore Siena, Andrea Sartore-Bianchi, Federica Di Nicolantonio, Julia Balfour, Alberto Bardelli

The monoclonal antibodies panitumumab and cetuximab that target the epidermal growth factor receptor (EGFR) have expanded the range of treatment options for metastatic colorectal cancer. Initial evaluation of these agents as monotherapy in patients with EGFR-expressing chemotherapy-refractory tumors yielded response rates of approximately 10%. The realization that detection of positive EGFR expression by immunostaining does not reliably predict clinical outcome of EGFR-targeted treatment has led to an intense search for alternative predictive biomarkers. Oncogenic activation of signaling pathways downstream of the EGFR, such as mutation of *KRAS*, *BRAF*, or *PIK3CA* oncogenes, or inactivation of the *PTEN* tumor suppressor gene is central to the progression of colorectal cancer. Tumor *KRAS* mutations, which may be present in 35%–45% of patients with colorectal cancer, have emerged as an important predictive marker of resistance to panitumumab or cetuximab treatment. In addition, among colorectal tumors carrying wild-type *KRAS*, mutation of *BRAF* or *PIK3CA* or loss of PTEN expression may be associated with resistance to EGFR-targeted monoclonal antibody treatment, although these additional biomarkers require further validation before incorporation into clinical practice. Additional knowledge of the molecular basis for sensitivity or resistance to EGFR-targeted monoclonal antibodies will allow the development of new treatment algorithms to identify patients who are most likely to respond to treatment and could also provide rationale for combining therapies to overcome primary resistance. The use of *KRAS* mutations as a selection biomarker for anti-EGFR monoclonal antibody (eg, panitumumab or cetuximab) treatment is the first major step toward individualized treatment for patients with metastatic colorectal cancer.

J Natl Cancer Inst 2009;101:1308–1324

The epidermal growth factor receptor (EGFR), a member of the human epidermal growth factor receptor (HER)–erbB family of receptor tyrosine kinases, represents an important target for cancer treatment because its activation stimulates key processes involved in tumor growth and progression, including proliferation, angiogenesis, invasion, and metastasis. The binding of EGF or other ligands to EGFR initiates a mitogenic signaling cascade via several pathways, including the RAS–RAF–mitogen-activated protein kinase (MAPK), phosphatidylinositol 3-kinase (PI3K)–Akt, and phospholipase Cγ pathways (1,2). Overexpression of EGFR is found in a range of solid tumor types and has been linked to poorer outcomes (3,4).

EGFR inhibitors—monoclonal antibodies targeting the extracellular domain and small-molecule tyrosine kinase inhibitors—have expanded the range of treatment options for various solid tumors. EGFR-targeted monoclonal antibodies have been extensively studied in metastatic colorectal cancer (Table 1), whereas tyrosine kinase inhibitors have thus far shown little activity in this setting (5,6). Cetuximab (ER-K0034, Erbitux, Merck-Serono KgaA, Darmstadt, Germany; ImClone Systems Inc, New York, NY), the first anti-EGFR monoclonal antibody to be approved for clinical use for metastatic colorectal cancer, is a chimeric mouse–human monoclonal antibody that has been evaluated primarily in combination with chemotherapy (7–10) but also as monotherapy (7,11,12). Panitumumab (ABX-EGF, Vectibix; Amgen Inc, Thousand Oaks, CA), a fully human monoclonal antibody, has shown efficacy as monotherapy in chemotherapy-refractory patients with metastatic colorectal cancer (13), and ongoing chemotherapy combination trials in earlier lines of treatment have reported acceptable interim safety data (14,15). In addition, cetuximab and panitumumab have both been evaluated in combination with bevacizumab, a monoclonal antibody targeting the vascular endothelial growth factor (VEGF), plus standard first-line chemotherapy (16,17). However, increased toxicity and a shorter progression-free interval were observed in the experimental groups compared with the control groups. Thus, the strategy of combining both an EGFR inhibitor and a VEGF inhibitor with chemotherapy appears to be detrimental and is not being pursued further.

Affiliations of authors: The Falck Division of Medical Oncology, Department of Oncology, Ospedale Niguarda Ca' Granda, Milan, Italy (SS, AS-B); Laboratory of Molecular Genetics, Institute for Cancer Research and Treatment, University of Torino Medical School, Turin, Italy (FDN, AB); Kilconquhar, Fife, Scotland (JB); Fondazione Italiana Ricerca Cancro Institute of Molecular Oncology, Milan, Italy (AB).

Correspondence to: Salvatore Siena, MD, The Falck Division of Medical Oncology, Ospedale Niguarda Ca' Granda, Piazza Ospedale Maggiore 3, 20162 Milan, Italy (e-mail: salvatore.siena@ospedaleniguarda.it); Alberto Bardelli, PhD, Laboratory of Molecular Genetics, Institute for Cancer Research and Treatment, University of Torino Medical School, Strada Provinciale 142, Km 3.95, 10060 Candiolo, Turin, Italy (e-mail: a.bardelli@unito.it).

See "Funding" and "Notes" following "References."

DOI: 10.1093/jnci/djp280

Advance Access publication on September 8, 2009.

Table 1. Anti–epidermal growth factor receptor (EGFR) monoclonal antibodies (mAbs) used for treatment of metastatic colorectal cancer (mCRC)

Agent	Description	Company	Approved indications	Investigational indications
Cetuximab (Erbitux)	Chimeric mAb	Merck-Serono KGaA, Darmstadt, Germany; ImClone Systems Inc, New York, NY	Treatment of patients with EGFR-expressing, *KRAS* wild-type mCRC in combination with chemotherapy (EU) or irinotecan in irinotecan-refractory disease (US) or as a single agent in patients who have failed oxaliplatin- and irinotecan-based therapy or who are intolerant to irinotecan (EU, US)	In combination with other targeted agents
Panitumumab (Vectibix)	Fully human mAb	Amgen Inc, Thousand Oaks, CA	Monotherapy for fluoropyrimidine-, oxaliplatin-, and irinotecan-resistant EGFR-expressing mCRC with wild-type *KRAS*	In combination with chemotherapy and/or other targeted agents

Cetuximab and panitumumab appear to have similar efficacy, achieving fairly modest but clinically meaningful objective response rates of approximately 10% when used as monotherapy for chemotherapy-refractory EGFR-expressing metastatic colorectal cancers (7,11–13,18). However, panitumumab is likely to be less immunogenic than cetuximab because of its fully human composition and, indeed, panitumumab seldom gives rise to severe infusion reactions (13). Such events may occur in up to 22% of cetuximab-treated patients, depending on geographical region (19,20), and appear to be commonly associated with preexisting specific IgE antibodies against the oligosaccharide component of the cetuximab molecule, galactose-α-1,3-galactose (21).

Positive EGFR protein expression, as determined by immunohistochemistry, was initially selected as an entry criterion for studies evaluating EGFR inhibitors on the assumption that sensitivity to such agents was associated with EGFR expression. However, a large body of evidence from patients who were treated with monoclonal antibodies for metastatic colorectal cancer (7,11,13,22,23) or tyrosine kinase inhibitors for other solid tumors (24,25) indicates that this biomarker is poorly associated with response to EGFR inhibitors in the clinical setting. Objective responses have been observed in patients with low or negative, as well as high, EGFR protein expression, as determined by immunohistochemistry. These findings have led to intense research to identify alternative predictive molecular biomarkers that can be used to identify patients who are most likely to benefit from EGFR-targeted treatment. This review discusses progress made toward these ends with a focus on treatment of metastatic colorectal cancer with anti-EGFR monoclonal antibodies.

Literature was identified in PubMed and oncology conference databases by use of the search terms "colorectal cancer" and "molecular markers" and retrieved articles were evaluated by the authors. All fully published clinical data relating to clinical response to treatment with monoclonal antibodies in metastatic colorectal cancer were considered for inclusion, as well as key conference abstracts. Additional searches of the same databases were performed to identify suitable background information.

Predicting Response: Molecular Biomarkers

Early work exploring molecular biomarkers of response to EGFR-targeted monoclonal antibodies (ie, cetuximab or panitumumab) as alternatives to EGFR protein determined by immunohistochemistry started in 2005 (26) and was based on retrospective analyses of archived tumor tissue from subsets of patients participating in clinical trials. However, more recently studies have been designed to incorporate biomarker analysis (eg, the pivotal phase III panitumumab study) (13,27). In general, primary tumor tissue was analyzed, although metastatic tissue was evaluated in some instances. In the majority of studies, cetuximab was given in combination with chemotherapy, which could make interpretation difficult, whereas to date, panitumumab has been administered almost exclusively as monotherapy. Although most biomarker datasets are from chemotherapy-refractory or relapsed patients who had received multiple previous lines of treatment, first-line data have recently been presented (28,29). Finally, it should also be mentioned that many analyses were based on objective responses alone, without taking disease stabilization into account.

Markers Downstream of EGFR

A rapidly growing body of knowledge has indicated that growth of many tumors is driven by constitutive activation of signaling pathways downstream of the EGFR, as will be discussed below. Figure 1 shows the interactions between various signaling pathways involved in tumor proliferation and progression. Such close interactions between these pathways may provide "escape mechanisms" that allow tumors to circumvent a pathway that has been pharmacologically blocked.

The interlinked RAS–MAPK and PI3K signaling pathways (Figure 1) play an important role in tumorigenesis via phosphorylation of various proteins and transcription factors that directly control cell growth, differentiation, and apoptosis (1,2,30). *KRAS*, a member of the rat sarcoma virus (*ras*) gene family of oncogenes (including *KRAS*, *HRAS*, and *NRAS*), encodes the guanosine diphosphate (GDP)– and guanosine triphosphate (GTP)–binding protein RAS that acts as a self-inactivating intracellular signal transducer (31). After binding and activation by GTP, RAS recruits the oncogene RAF, which phosphorylates MAP2K (mitogen-activated protein kinase kinase)-1 and MAP2K-2, thus initiating MAPK signaling that ultimately leads to expression of proteins playing important roles in cell growth, differentiation, and survival. The oncogene *PIK3CA* encodes the p110 subunit of PI3K, which can be activated via interaction with RAS proteins (1,2,30).

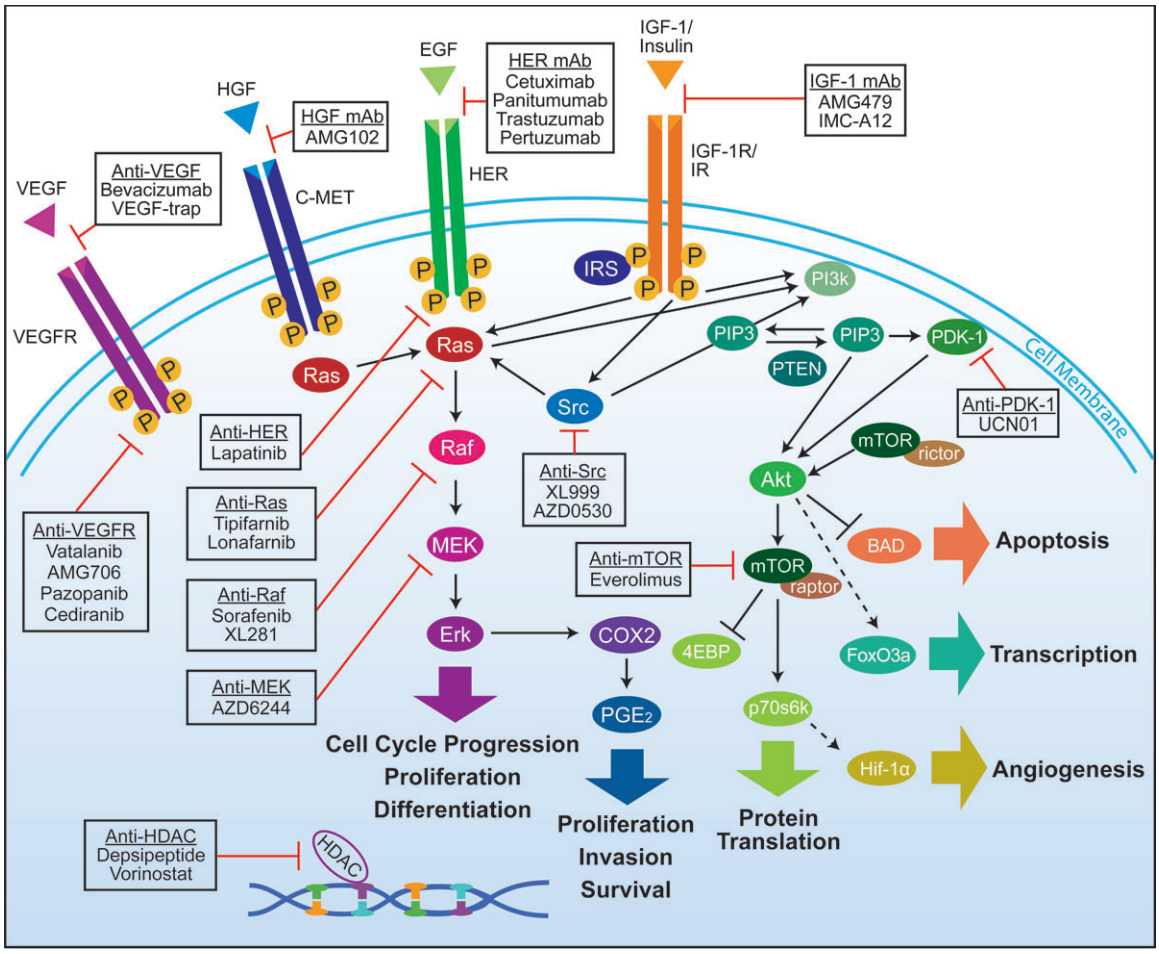

Figure 1. Overview of interlinked cellular signaling pathways involved in the proliferation and progression of colorectal cancer. Agents targeting signaling proteins that have been evaluated or are currently being evaluated in phase II, III, or IV clinical trials for colorectal cancer are shown. The epidermal growth factor receptor (EGFR)–related family of receptor tyrosine kinases includes human epidermal growth factor receptor (HER1), EGFR, or c-erbB1; HER2 or c-erbB2; HER3 or c-erbB3; and HER4 or c-erbB4. C-MET = mesenchymal–epithelial transition factor; EGF = epidermal growth factor; HDAC = histone deacetylases; HGF = hepatocyte growth factor; IGF-1 = insulin-like growth factor-I; IGF-1R = insulin-like growth factor-I receptor; IR = insulin receptor; VEGF = vascular endothelial growth factor; VEGF-R = vascular endothelial growth factor receptor.

Mutation in *KRAS*, *BRAF*, or *PIK3CA* results in continuous activation of the downstream RAS–MAPK or PI3K pathways, regardless of whether the EGFR is activated or pharmacologically blocked. Such activation in turn enhances transcription of various oncogenes, including *MYC*, *CREB*, and the gene for nuclear factor κB (1,2,30).

A recent population-based study of 586 patients with colon adenocarcinomas found mutations in *KRAS*, *BRAF*, and/or *PIK3CA* in 316 (56%) of the 586 tumors studied (32). *KRAS* is the most commonly mutated gene in this pathway, with mutations in 35%–45% of colorectal adenocarcinomas; mutations in *PIK3CA* (≤20%) and *BRAF* (<15%) are less common (32–37). Mutations in *PIK3CA* and *KRAS* or *BRAF* may coexist within the same tumor (32,36–38), but *KRAS* and *BRAF* mutations appear to be mutually exclusive (33,34,39–41). *KRAS* mutation is thought to be an early event in tumorigenesis (42,43), and, in general, metastatic and primary sites have been concordant with regard to *KRAS* status (44–46), with only small differences having been reported (47,48). *KRAS* mutations have been explored as prognostic biomarkers (independent of anti-EGFR monoclonal antibody treatment), but data are conflicting, reflecting differences in datasets and methodologies and possibly tumor heterogeneity (32,43,49–54). Retrospective data from 2721 patients with colorectal cancer from the RASCAL (ie, the Kirsten ras in Colorectal Cancer Collaborative Group) study (43) indicated that *KRAS* mutations may be associated with increased risk of death ($P = .002$). However, in phase III monotherapy studies of cetuximab (55) or panitumumab (13,27), *KRAS* mutations did not appear to affect outcome among patients receiving only best supportive care. Furthermore, *KRAS* mutations do not appear to have a stage-specific prognostic value: No association between tumor *KRAS* mutations and relapse-free survival was observed among patients with stage II and stage III colorectal cancer in the Pan-European Trials in Adjuvant Colon Cancer (PETACC) 3 study (54).

KRAS Mutations. A number of groups undertook retrospective testing of *KRAS* status of tumors from patients with metastatic colorectal cancer who were treated with cetuximab or panitumumab (with or without chemotherapy) (26,33,34). Lievre et al. (34) first reported the link between *KRAS* mutations and lack of response to EGFR-targeted monoclonal antibodies, a concept

previously proposed by Moroni et al. (26), based on their cohort study (n = 30 patients). These findings were confirmed and extended to *BRAF* in a series of 48 patients by Benvenuti et al. (33), who also found that transfection of mutated *KRAS* (G12V) into wild-type DiFi colorectal cancer cells confers resistance to cetuximab. *KRAS* mutations have since emerged as a major predictor of resistance to panitumumab or cetuximab in the clinical setting. Studies (27,34,36,55–60) of patients receiving first and subsequent lines of treatment have found that those with tumors carrying *KRAS* mutations do not respond to EGFR-targeted monoclonal antibodies or experience any survival benefit from such treatment. Indeed, the progression-free interval in patients with tumors carrying mutant *KRAS* generally appears to be approximately half that of those patients whose tumors carry wild-type *KRAS* (Table 2).

The pivotal randomized phase III study of panitumumab monotherapy in the relapsed or refractory setting (13) was the first large study (n = 463 patients) to confirm the negative predictive value of *KRAS* mutations (27). Biomarker analysis of primary tumor tissue was planned in the protocol and *KRAS* analysis was performed in a blinded manner at a central laboratory by use of a *KRAS* testing kit (DxS Ltd, Manchester, UK) (27). Among the 463 patients enrolled in this study, 427 (92%) were included in the *KRAS* analysis. Of these 427, 184 (43%) were found to have tumors harboring mutant *KRAS*: 84 (40%) of the 208 patients randomly assigned to panitumumab plus best supportive care and 100 (46%) of the 219 patients assigned to best supportive care alone. Among the 208 patients assigned to panitumumab, 21 (17%) of the 124 patients in the wild-type *KRAS* subgroup achieved objective response, whereas none of the 84 patients in the mutant *KRAS* subgroup responded to this treatment. Median progression-free interval among those treated with panitumumab was 12.3 weeks among those in the wild-type *KRAS* subgroup and 7.4 weeks among those in the mutant *KRAS* subgroup. The hazard ratio (HR) for disease progression or death (panitumumab vs control group) was 0.45 (95% confidence interval [CI] = 0.34 to 0.59) for panitumumab in the wild-type *KRAS* subgroup, but there was no benefit of panitumumab in the mutant *KRAS* subgroup (HR = 0.99, 95% CI = 0.73 to 1.36) (Figure 2). A sensitivity analysis that adjusted for potential bias from unscheduled assessment found similar results. A total of 168 (77%) of the 219 *KRAS*-evaluable patients initially assigned to the control group crossed over to receive panitumumab after disease progression, at a median time of 7.1 weeks; this crossover confounded analysis of overall survival. Among these 168 patients, 20 (22%) of the 91 in the wild-type *KRAS* subgroup, compared with none in the mutant *KRAS* subgroup, responded to panitumumab treatment; it is important to note that these results were based on local review, whereas tumor response in the main study was based on central review (27).

KRAS data from three large randomized phase II–III cetuximab studies have recently been published, including the first-line OPUS (ie, Oxaliplatin and Cetuximab in First-Line Treatment of metastatic colorectal cancer) (28) and CRYSTAL (ie, Cetuximab Combined With Irinotecan in First-Line Therapy for Metastatic Colorectal Cancer) (29) studies and the NCIC-CTG (ie, National Cancer Institute of Canada-Clinical Trials Group) monotherapy study conducted in relapsed or refractory patients or those with contraindications to chemotherapy (55). Data from 394 (69%) of the 572 *KRAS*-evaluable patients participating in the phase III NCIC–CTG trial confirmed that patients with tumors carrying *KRAS* mutations do not benefit from cetuximab monotherapy. Both progression-free and overall survival were similar for the cetuximab and control groups in those patients with tumors carrying *KRAS* mutations (progression-free interval = 1.8 vs 1.8 months [HR = 0.99, 95% CI = 0.73 to 1.35, $P = .96$]; overall survival = 4.6 vs 4.5 months [HR = 0.98, 95% CI = 0.70 to 1.37, $P = .89$]). However, in the subgroup whose tumors carried wild-type *KRAS*, cetuximab treatment was associated with statistically significantly ($P < .001$) longer survival than control treatment (progression-free interval = 3.7 vs 1.9 months [HR = 0.40, 95% CI = 0.30 to 0.54]; overall survival = 9.5 vs 4.8 months [HR = 0.55, 95% CI = 0.41 to 0.74, $P < .001$]) (55). It should be noted that unlike the pivotal panitumumab phase III study (13), the design of this study did not allow patients from the control group who had disease progression to cross over to monoclonal antibody treatment. Final retrospective data from the OPUS and CRYSTAL studies indicate that the addition of cetuximab to first-line FOLFOX (folinic acid, fluorouracil, and oxaliplatin) (28) or FOLFIRI (folinic acid, fluorouracil, and irinotecan) (29) chemotherapy does not benefit patients with tumors carrying *KRAS* mutations, although those patients can benefit from chemotherapy alone (Table 2). Indeed, findings of the OPUS study indicate that addition of EGFR-targeted treatment to chemotherapy may even be detrimental in such patients (28) (Table 2).

In the PACCE (ie, Panitumumab Advanced Colorectal Cancer Evaluation) study (16), adding panitumumab to bevacizumab and chemotherapy was associated with shortening of the progression-free interval among patients with tumors carrying wild-type *KRAS* (11.5 months in the chemotherapy–bevacizumab arm vs 9.8 months in the panitumumab-chemotherapy–bevacizumab arm). In the CAIRO (ie, Capecitabine, Irinotecan, and Oxaliplatin trial)–2 study (17), addition of cetuximab to capecitabine, oxaliplatin, and bevacizumab as first-line treatment in patients with metastatic colorectal cancer had no effect on progression-free interval among those with tumors carrying wild-type *KRAS* (10.6 months in the chemotherapy–bevacizumab arm vs 10.5 months in the combined cetuximab arm). However, this combination had a marked detrimental effect among patients with tumors carrying mutated *KRAS* (12.5 vs 8.1 months) (17).

The proportion of patients bearing wild-type *KRAS* tumors who fail to achieve either objective response or disease stabilization with panitumumab or cetuximab varies considerably between studies (Table 2). Among a total of 124 patients with such tumors who were treated with panitumumab in the pivotal phase III study, 45 (36%) had a best response of progressive disease (61). These patients had a median progression-free interval of 7.3 weeks, as shown by a post hoc subanalysis (Figure 3). However, among patients with stable disease or a partial response, median progression-free interval was 23.9 and 27.0 weeks, respectively (61).

It is interesting to note that in other solid tumor settings, EGFR inhibitors (tyrosine kinase inhibitors or cetuximab) have shown minimal activity in patients with pancreatic cancer (62,63), which is associated with a very high prevalence of *KRAS* mutations (approximately 90%) (64), and in patients with lung cancer whose tumors carry *KRAS* mutations (65–67).

Table 2. Tumor *KRAS* mutations and outcome of panitumumab- or cetuximab-based treatment in patients with metastatic colorectal cancer*

First author (reference)	Treatment [type of study; type of patients]	No. of patients with *KRAS* mutation/total No. of patients (%)	Outcome by *KRAS* status, No. of patients (%)†						Association of *KRAS* mutation with response and survival parameters
			Complete or partial response		Stable disease		Progressive disease		
			MT	WT	MT	WT	MT	WT	
Monotherapy									
Amado (27)‡*	Panitumumab, [phase III, chemotherapy refractory]	84/208 (40)	0/84 (0)	21/124 (17)	10 (12)	42 (34)	59 (70)	45 (36)	*KRAS* mutation associated with shorter PFS vs wild type (7.4 vs 12.3 wk): no benefit of panitumumab vs BSC in this subgroup
Amado (27)§	Panitumumab crossover [phase III extension, chemotherapy refractory]	77/168 (46)	0/77 (0)	20/91 (22)	20 (26)	35 (38)	37 (48)	23 (25)	
Freeman (36)	Panitumumab [patient cohort, chemotherapy refractory]	24/62 (39)	0/24 (0)	4/38 (11)	5 (21)	20 (53)	19 (79)	14 (37)	Median PFS = 7.4 wk for MT *KRAS* vs 16.2 wk for WT. Median OS = 22.2 wk for MT *KRAS* vs 42.9 wk for WT
Karapetis (55)	Cetuximab [phase III, chemotherapy refractory]	81/198 (41)	1/81 (1)	15/117 (13)	NA	NA	NA	NA	*KRAS* mutation associated with shorter PFS and OS vs WT ($P < .001$ for both)
Khambata-Ford (59)*	Cetuximab [NA, chemotherapy refractory]	30/80 (38)	0/30 (0)	5/50 (10)	3 (10)	19 (38)	27 (90)	26 (52)	*KRAS* mutations found in three (11%) DC vs 27 (51%) NR ($P < .001$) and associated with lower DC rate (10% vs 48%; $P < .001$) but similar PFS (59 vs 61 d)
Mainly combination therapy									
Benvenuti (33)	Cetuximab ± CT or panitumumab [patient cohort, chemotherapy naïve and refractory]	16/48 (33)	1/16 (6)	10/32 (31)	5 (31)	8 (25)	10 (63)	14 (44)	*KRAS/BRAF* mutation negatively associated with PR ($P < .01$). *KRAS* mutation associated with shorter TTP ($P = .04$)
De Roock (57)	Cetuximab ± CT [patient cohort, chemotherapy refractory]	42/108 (39)	0/42 (0)	27/66 (41)	31 (74)	28 (42)	11 (26)	11 (17)	*KRAS* mutation found in 0% of OR vs 52% of NR ($P < .001$) and associated with shorter OS (27.3 vs 43.0 wk ($P = .02$)
Di Fiore (56)	Cetuximab + CT [patient cohort, chemotherapy refractory]	22/59 (37)	0/22 (0)	12/37 (32)	5 (23)	14 (38)	17 (77)	11 (30)	*KRAS* mutation was associated with PD ($P < .001$) and shorter TTP (3 vs 5.5 mo; $P < .01$)
Lievre (34)	Cetuximab ± CT [patient cohort, chemotherapy naïve and refractory]	13/30 (43)	0/13 (0)	11/17 (65)	4 (31)	2 (12)	9 (69)	4 (24)	*KRAS* mutation associated with shorter OS (6.9 vs 16.3 mo; $P = .02$)
Lievre (60)	Cetuximab + CT [patient cohort, chemotherapy refractory]	24/89 (27)	0/24 (0)	26/65 (40)	NA	NA	NA	NA	*KRAS* mutation associated with shorter PFS (10.1 vs 31.4 wk; $P < .001$) and OS (10.1 vs 14.3 mo; $P = .03$)

(Table continues)

Table 2 (continued).

First author (reference)	Treatment [type of study; type of patients]	No. of patients with *KRAS* mutation/total No. of patients (%)	Outcome by *KRAS* status, No. of patients (%)†						Association of *KRAS* mutation with response and survival parameters
			Complete or partial response		Stable disease		Progressive disease		
			MT	WT	MT	WT	MT	WT	
Combination with chemotherapy (first-line setting)									
Bokemeyer (28)	FOLFOX [phase II, chemotherapy naïve]	47/233 (20)	23/47 (49)	27/73 (37)	17/47 (36)	30/73 (41)	5/47 (11)	12/73 (16)	Median PFS = 8.6 vs 7.2 mo in MT and WT patients, respectively
	FOLFOX + cetuximab [phase II, chemotherapy naïve]	52/233 (22)	17/52 (33)	37/61 (61)	27/52 (52)	19/61 (31)	7/52 (13)	3/61 (5)	No benefit of adding cetuximab to FOLFOX in *KRAS* MT patients, median PFS = 5.5 vs 7.7 mo in MT and WT patients, respectively
Van Cutsem (29)	FOLFIRI [phase III, chemotherapy naïve]	87/263 (33)	(40)	(43)	(46)	(44)	NA	NA	Median PFS = 8.1 vs 8.7 mo in MT and WT patients, respectively; median OS = 17.7 vs 21.0 mo in MT and WT patients, respectively
	FOLFIRI + cetuximab [phase III, chemotherapy naïve]	105/277 (38)	(36)	(59)	(47)	(31)	NA	NA	No benefit of adding cetuximab to FOLFIRI in *KRAS* MT patients, median PFS = 7.6 vs 9.9 mo in MT and WT patients, respectively; median OS = 17.5 vs 24.9 mo in MT and WT patients, respectively

* Studies that prospectively evaluated biomarkers. BSC = best supportive care; CR = complete response; CT = chemotherapy; DC = disease control (PR or SD); FOLFIRI = folinic acid, fluorouracil, and irinotecan; FOLFOX = folinic acid, fluorouracil, and oxaliplatin; MT = mutant; NA = not available; NR = nonresponse or nonresponder; OR = objective response or responder; OS = overall survival; PD = progressive disease; PFS = progression-free interval; PR = partial response or responder; SD = stable disease; TTP = time to disease progression; WT = wild type.

† Expressed as a percentage of patients within the MT and WT subgroup. (the denominator is also shown in the first two columns).

‡ Phase III comparison of panitumumab vs BSC (data for panitumumab recipients only are shown).

§ Patients initially assigned to BSC who crossed over to panitumumab treatment after disease progression in the phase III study.

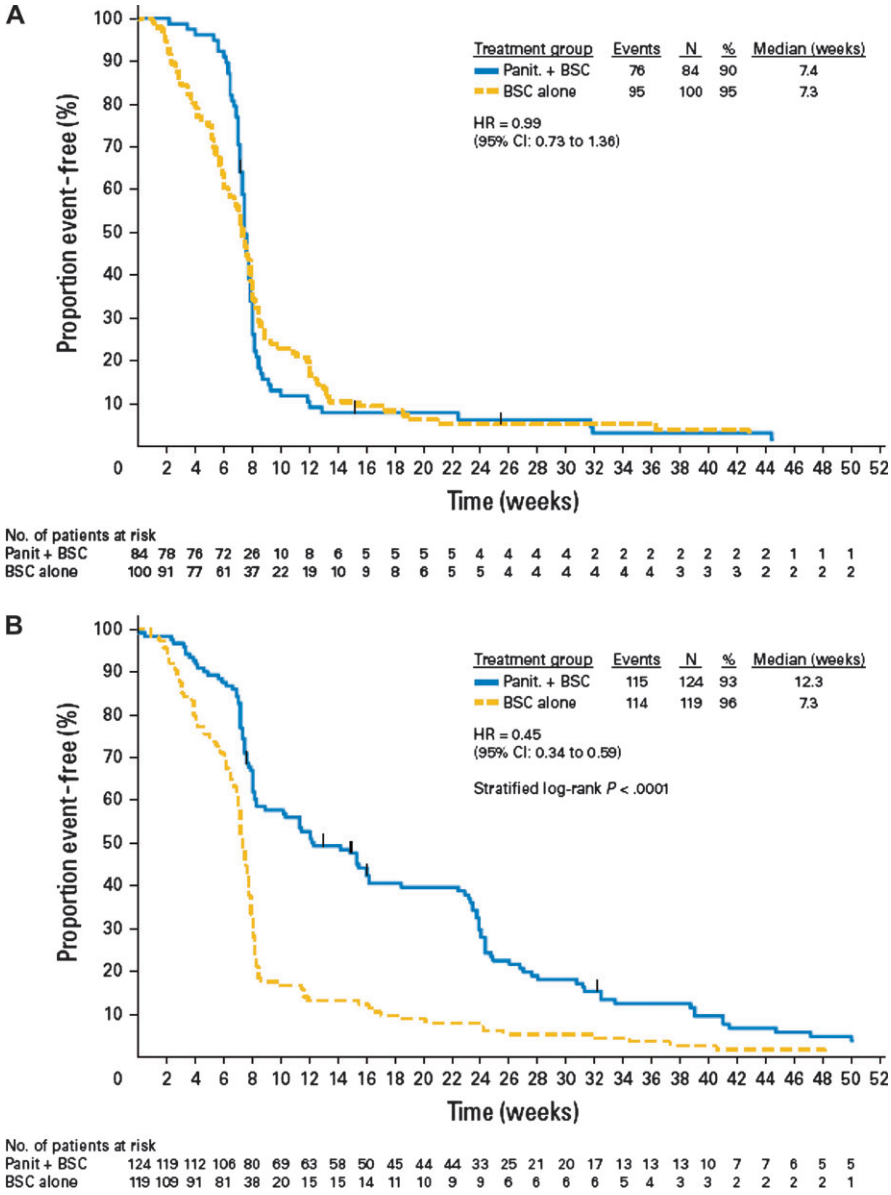

Figure 2. Progression-free interval and *KRAS* mutation status of tumor in patients with metastatic colorectal cancer who were randomly assigned to treatment with best supportive care (BSC) alone or panitumumab (Panit) plus BSC in a phase III study (27). **A**) Tumors with mutant *KRAS* status. **B**) Tumors with wild-type *KRAS* status (27) (with permission from the American Society of Clinical Oncology). CI = confidence interval; HR = hazard ratio.

BRAF Mutations. Recently published retrospective analysis (40) of 113 tumors from patients who received panitumumab or cetuximab in second or subsequent lines of treatment showed that those with tumors that carried *BRAF* V600E mutations (n = 11, 10%) did not respond to EGFR inhibition and had statistically significantly shorter progression-free interval ($P = .001$) and overall survival ($P < .001$) than patients whose tumors carried wild-type *BRAF* (n = 34). A similar pattern was observed in an analysis of 231 tumors from patients treated with first-line cetuximab plus capecitabine, oxaliplatin, and bevacizumab in the CAIRO-2 study (41). The median progression-free interval and overall survival were 6.6 and 15.2 months for patients with tumors carrying mutant *BRAF* (n = 28), vs 10.4 ($P = .01$) and 21.5 ($P = .001$) months in those with tumors carrying wild-type *BRAF* (n = 231). However, the response rate did not differ between these two patient subgroups (39% vs 48%; $P = .04$).

Di Nicolantonio et al. (40) also demonstrated that introduction of the *BRAF* V600E allele could confer resistance to either cetuximab or panitumumab in wild-type *BRAF* colorectal cancer cells. Furthermore, they showed that the multikinase inhibitor sorafenib may restore sensitivity to EGFR inhibitors in *BRAF*-mutated colorectal cancer cell lines. Consequently, combined sorafenib and cetuximab therapy is undergoing clinical evaluation in metastatic colorectal cancer in a National Cancer Institute–sponsored trial (NCT00343772; http://clinicaltrials.gov/ct2/show/NCT00343772). Evaluation of EGFR-targeted antibodies in combination with other novel and more selective *BRAF* inhibitors, such as PLX-4032 and XL-281, is also warranted.

PTEN and PI3K. Loss of expression of the tumor suppressor PTEN protein, which regulates the PI3K–Akt signaling pathway, has been reported to confer tumor resistance to EGFR tyrosine kinase inhibitors in vitro (68) and has been linked to erlotinib resistance in patients with glioblastoma (69) and to trastuzumab resistance in patients with breast cancer (70). In vitro studies in various colon cancer cell lines have found that activating *PIK3CA* mutations or loss of PTEN expression

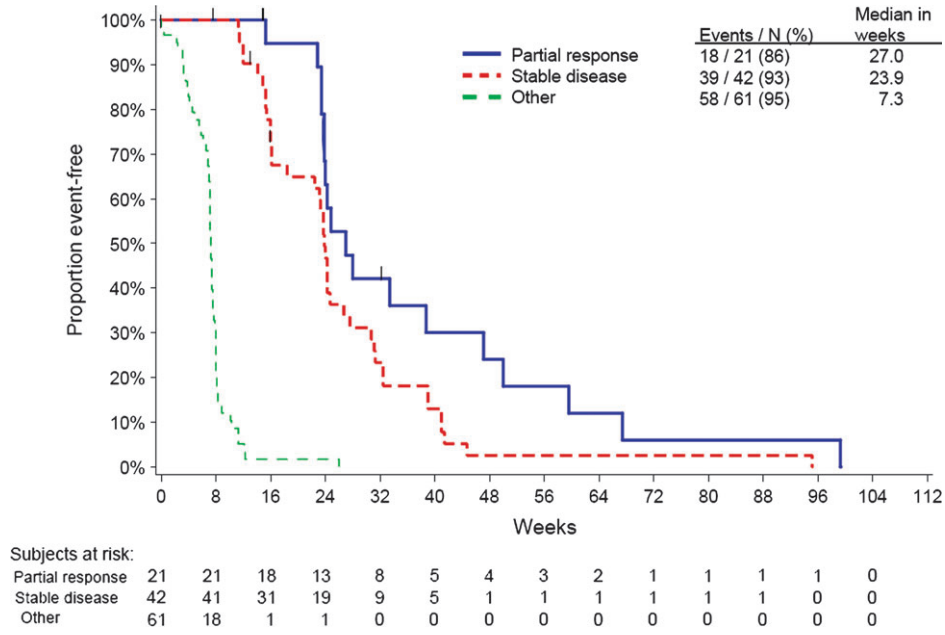

Figure 3. Progression-free interval by response to panitumumab in a subgroup of patients with metastatic colorectal cancer whose tumors carry wild-type *KRAS*. Data are from a phase III study (27,61).

appeared to confer resistance to cetuximab. Cell lines that had mutations in *PIK3CA*, or were PTEN null, and also had mutations in *RAS* or *BRAF* exhibited the greatest resistance to cetuximab (71). Similarly, in the clinical setting, deregulation of either PIK3CA or *PTEN* gene (via mutation or loss of expression; $P = .02$) (38) or PTEN protein expression loss alone ($P < .001$) (58) statistically significantly impaired response to cetuximab-based treatment in colorectal cancer patients. Frattini et al. (58) reported that none of 11 patients with tumor PTEN loss responded to cetuximab-based treatment, whereas 10 (63%) of 16 patients with intact PTEN protein expression were partial responders. Perrone et al. (38) also noted that none of three patients with *PTEN* mutation responded to treatment with cetuximab and irinotecan. In a total of 92 patients with metastatic colorectal cancer who were included in three biomarker analyses (26,34,38), nine (10%) had tumors bearing *PIK3CA* mutations and only one responded to EGFR-targeted treatment. In a larger patient series (n = 110), Sartore-Bianchi et al. (72) found that *PIK3CA* mutations and PTEN loss in colorectal tumors were statistically significantly associated with lack of response to panitumumab (zero of 15 patients, $P = .038$) or cetuximab (one of 32 patients, $P = .001$) treatment. In the same study, *PIK3CA* mutations and/or loss of PTEN expression were negatively associated with progression-free interval, and loss of PTEN expression was also linked with poorer overall survival ($P = .005$). These investigators suggested that combining mutation analysis for *KRAS* and *PIK3CA* (loss of *PTEN* and/or *PIK3CA* mutation) could identify up to 70% of patients with metastatic colorectal cancer who are unlikely to respond to treatment with an EGFR-targeted monoclonal antibody (72). Contradictory evidence was recently reported by Prenen et al. (73) who found no strong rationale for using *PIK3CA* mutations as a single marker for sensitivity to cetuximab in chemotherapy-refractory metastatic colorectal cancer. Razis et al. (74) reported that normal PTEN protein expression was associated with a higher response rate and longer time to progression in patients treated with cetuximab-based therapy, despite a 50% response rate observed in patients who had lost PTEN protein expression (74). Because this study included patients treated with first-line cetuximab and chemotherapy, these findings are difficult to interpret. Further investigation and prospective data from large randomized clinical trials are required to confirm these findings before they can be integrated into clinical practice.

Because tumors with oncogenic *PIK3CA* mutations are likely to be driven by PI3K as the primary source of growth, proliferation, and survival signaling (Figure 1), the use of selective PI3K inhibitors is being tested in ongoing trials. Indeed, several PI3K inhibitors are progressing from preclinical development to phase I clinical studies. This family of compounds includes XL147, GDC-0941, BGT226, and the pan-PI3K–mammalian target of rapamycin (mTOR) inhibitors XL765 and NVP-BEZ235 (75). The combination of cetuximab and the mTOR inhibitor everolimus has been shown to be effective in restoring growth inhibition in cetuximab-resistant colorectal cancer cells (76). It remains to be evaluated in clinical trials whether concomitant inhibition of the EGFR and PIK3CA pathways (Figure 1) will convey clinical benefit.

In addition, in colorectal cancer, the incidence of *PIK3CA* and *BRAF* mutations (but not mutations of *KRAS* or *TP53*) displays a gender bias with a higher frequency occurring in women (32,77). Thus, it could be hypothesized that female patients with metastatic colorectal cancer might be less likely to benefit from treatment with EGFR-targeted monoclonal antibodies. However, available clinical data do not support this hypothesis (17,27,29).

EGFR as a Target

Many technical reasons have been advocated for the lack of association between EGFR detection by immunohistochemistry and response to EGFR-targeted treatment, as discussed by Shia et al. (78). These reasons include disparity between the form or epitope of EGFR detected by immunohistochemistry and that targeted by

anti-EGFR monoclonal antibodies, as well as issues related to processing and handling of tumor tissue samples, such as prolonged storage. Immunohistochemistry is also a semiquantitative method that lacks a standardized scoring system and is subject to interobserver variation. Moreover, differences between primary colorectal tumors and their metastases with regard to EGFR expression have been reported (79), indicating that reliance on such biomarkers in the primary tumor to predict treatment response of metastatic growths may be inappropriate.

EGFR Affinity and Phosphorylation. Using a specific ligand-binding assay, Francoual et al. (80) found that many tumors contain both low- and high-affinity EGFRs: 64 (78%) of 82 tumor specimens contained only high-affinity binding sites (median dissociation constant = 0.75 nM) and 18 (22%) had both low- and high-affinity sites. Because immunohistochemistry-based methods cannot distinguish between low- and high-affinity EGFR, these findings may provide further explanation for the lack of correlation between EGFR immunostaining and clinical response to EGFR-targeted treatment.

EGFR phosphorylation status may reflect the level of receptor utilization by the tumor. This parameter (as determined by immunohistochemistry) was associated with clinical response in patients treated with cetuximab-based therapy. Patients with an activated or phosphorylated EGFR score, as indicated by an immunohistochemistry-based visual score of 7 or greater, were almost twice as likely to have disease control (objective response or stable disease) than those with a score of less than 7 (100% vs 54%; $P = .05$) (81).

EGFR Gene Status. Activating mutations, including in-frame deletions and amino acid substitutions in exons 18, 19, and 21 in the *EGFR* catalytic domain, play an important role in determining responsiveness to tyrosine kinase inhibitors in the lung cancer setting (82,83). Such mutations are rare (26,84) or absent (57,85) in colorectal cancer tumors. Moroni et al. (26) detected one mutation (3.2%) among 31 patients with metastatic colorectal cancer, occurring in a patient who achieved stable disease for 24 weeks with cetuximab and chemotherapy treatment. This missense heterozygous mutation in exon 21 (Gly857Arg) affected a residue located within the activation loop of the *EGFR* catalytic domain and was one amino acid away from the Leu858Arg-activating mutation that has been identified in patients with lung cancer who respond to gefitinib or erlotinib (86). At disease progression, the patient whose tumor had this mutation was treated with gefitinib; this molecular alteration in EGFR was not associated with clinical response because the disease progressed after 4 weeks of treatment. Notably, a specific polymorphism of *EGFR* affecting exon 13 at residue 521 Arg/Arg (previously identified as residue 497, rs11543848) has been linked with improved overall survival in women with metastatic colorectal cancer (vs Lys/Lys and/or Lys/Arg variants), although the reverse pattern was observed in men with this disease (87). This same polymorphism has been linked to cetuximab response in other studies (88–90). Conflicting evidence also exists for a polymorphism affecting the ligand of EGFR, EGF, at position 61 (rs4444903) (89,91,92).

A small proportion of colorectal tumors overexpress EGFR via amplification of the gene, which can be detected by fluorescence in situ hybridization (FISH) (93) or chromogenic in situ hybridization (78). Although the intensity of protein expression was associated with the likelihood of gene amplification, immunohistochemistry had a low specificity (17% in primary tumors and 23% in metastatic tumors) for predicting gene amplification (78).

When *EGFR* gene copy number was evaluated by polymerase chain reaction, no association was found between this parameter and clinical outcome of panitumumab- or cetuximab-based treatment (26,57,85), probably because of tumor DNA dilution by DNA from normal cells during DNA extraction. However, *EGFR* gene copy number as analyzed by FISH or chromogenic in situ hybridization appears to be a promising biomarker of response to such treatment (Table 3), and present technical limitations are being addressed in pathology studies (97). Methods of tissue processing and *EGFR* scoring systems differed between studies. Moreover, FISH pattern for *EGFR* expression is often nonhomogeneous in colorectal cancer tumors, with variable ratios of disomy vs polysomy or amplification being observed (93,94). Increased gene copy number was found in at least 30% of patients when a threshold value of approximately three *EGFR* copies per nucleus was used, as determined by FISH, compared with only 10% of patients when a threshold of six or more *EGFR* copies per nucleus was used, as determined by chromogenic in situ hybridization (Table 3). Statistically significant concordance between primary colorectal tumors and their metastases with regard to *EGFR* gene copy number has been found, as identified by FISH (95,96).

Available data suggest that patients with less than three *EGFR* gene copies per nucleus have a relatively low likelihood of responding to EGFR-targeted monoclonal antibody treatment (34,56,94–96). In a retrospective analysis of a subgroup of patients participating in the pivotal phase III trial of panitumumab monotherapy (94), the mean *EGFR* gene copy number per nucleus and the percentage of tumor cells with chromosome 7 polysomy (three or more *EGFR* signals per nucleus) were analyzed by FISH and the association between these parameters and clinical outcome was assessed. None of the patients with a mean of <2.47 *EGFR* gene copies per nucleus or fewer than 43% of tumor cells with chromosome 7 polysomy, respectively, achieved objective response compared with six (30%) of the 20 patients ($P = .001$) and six (32%) of the 19 patients ($P = .001$) who had values above these thresholds. A mean *EGFR* gene copy number threshold of less than 2.5 copies per nucleus or fewer than 40% of tumor cells with chromosome 7 polysomy discriminated patients with shorter progression-free ($P = .039$ and $P = .029$, respectively) and overall survival ($P = .015$ and $P = .014$, respectively). *EGFR* gene copy number and chromosome 7 polysomy status did not draw a parallel with progression-free interval in patients receiving only supportive care in this study, suggesting that this parameter is not prognostic in metastatic colorectal cancer (94). These data contrast with earlier findings that were based on quantitative polymerase chain reaction analysis (85) showing that *EGFR* gene copy number, as assessed by this method, related to neither clinical response nor progression-free interval. Homogeneous (ie, 100%) chromosome 7 disomy was the most common pattern found in 58 colorectal tumors with nonincreased gene copy number (n = 26; 45%) (94). Chromosome 7 disomy is easier to detect than an increase in *EGFR* gene copy number and, therefore, might enable a more reproducible FISH assay. However,

Table 3. Tumor epidermal growth factor receptor gene copy number and outcome of panitumumab- or cetuximab-based treatment in patients with metastatic colorectal cancer*

First author (reference)	Treatment [type of study; type of patients]	No of patients with increased GCN/total No. of patients (%) [cutoff†; methodology]	Outcome by GCN status, No. of patients (%)‡						Association of increased *EGFR* GCN with response and survival parameters
			Complete or partial response		Stable disease		Progressive disease		
			Increased	Normal	Increased	Normal	Increased	Normal	
Monotherapy									
Sartore-Bianchi (94)	Panitumumab [patient cohort, chemotherapy refractory]	20/58 (34) [≥2.47; FISH]	6/20 (30)	0/38 (0)	5 (25)	9 (24)	9 (45)	29 (76)	No OR if mean *EGFR* GCN of <2.47 copies per nucleus or <43% of tumor cells with chromosome 7 polysomy vs 6/20 (30%; $P < .001$) and 6/19 (32%; $P < .001$) among those with higher values. Mean *EGFR* GCN of <2.5 copies per nucleus or <40% of tumor cells with chromosome 7 polysomy associated with shorter PFS ($P = .0153$ and .0386, respectively) and OS ($P = .0145$ and .0290, respectively)
Other									
Cappuzzo (95)	Cetuximab ± CT [patient cohort, chemotherapy refractory]	43/85 (51) [2.92; FISH]	14/43 (33)	1/42 (2)	NA	NA	NA	NA	Increased *EGFR* GCN associated with higher OR ($P < .001$) and longer TTP (6.6 vs 3.5 mo, $P = .02$)
Personeni (96)	Cetuximab ± CT [patient cohort, chemotherapy refractory]	[≥2.83; FISH]	NA	NA	NA	NA	NA	NA	Longer PFS (5.5 vs 4.0 mo, $P = .25$) and OS (10 vs 8.3 mo, $P = .037$) in patients with mean GCN ≥2.83
Frattini (58)	Cetuximab ± CT [patient cohort, chemotherapy naïve and refractory]	8/27(30) [≥3 *EGFR*/CEP7; FISH] or 16/27 (59) [≥4.00 *EGFR*]	6/8 (75) or 4/16 (25)	0/3 (0)	0/8 (0) or 2/16 (12)	1/3 (33)	2/8 (25) or 10/16 (62)	2/3 (67)	Two patients with increased *EGFR* GCN had PD, possibly due to concomitant *KRAS* mutations. All NR with *EGFR* gene amplification or increased GCN also showed concomitant *KRAS* mutations and/or absent PTEN expression
Lievre (34)	Cetuximab ± CT [patient cohort, chemotherapy naïve and refractory]	3/30 (10)] [≥6; CISH]§	3/3 (100)	8/27 (30)	0 (0)	6 (22)	0 (0)	13 (48)	Increased *EGFR* GCN in 27% of OR vs 0% of NR ($P = .04$)
Moroni (26)	Panitumumab or cetuximab ± CT [patient cohort, chemotherapy naïve and refractory]	9/29 (31) [≥3; FISH]	8/9‖ (89)	1/20 (5)	0 (0)	5 (25)	1(11)	14 (70)	Increased *EGFR* GCN in 8/9 (89%) OR vs 1/20 (5%) NR ($P < .001$)

* CEP7 = chromosome 7 control; CISH = chromogenic in situ hybridization; CT = chemotherapy; EGFR = epidermal growth factor receptor; FISH = fluorescence in situ hybridization; GCN = gene copy number; NA = data not available; NR = nonresponders; OR = objective response or responder (ie, complete or partial response); OS = overall survival; PD = progressive disease; PFS = progression-free interval; PTEN = phosphatases and tensin homolog; TTP = time to disease progression.

† Expressed as number per nucleus.

‡ Expressed as a percentage of patients with increased or normal GCN (the denominator is also shown in the first two columns).

§ In more than 50% of cancer cells or presence of large gene copy cluster.

‖ High overall response rate in this study was due to a clinical enrichment strategy.

methods need to be further standardized for better reproducibility and optimum sensitivity (96,97).

In comparison with patients with normal *EGFR* gene copy number, patients with an increased *EGFR* gene copy number exhibit higher response rates to EGFR-targeted monoclonal antibodies, with a longer progression-free interval or time to progression (34,58,94–96) (Table 3). Two studies found that six (30%) of 20 patients and 14 (33%) of 43 patients with increased *EGFR* gene copy number had an objective response to panitumumab (94) or cetuximab with or without chemotherapy (95). Such response rates compare favorably with historical response rates reported for populations selected by EGFR immunostaining alone. Higher response rates were seen in other studies (Table 3). For instance, Moroni et al. (26) found an 89% response rate in a subgroup of nine patients with colorectal cancer whose tumors had an increased *EGFR* gene copy number, but these investigators included a relatively high proportion of responders (nine of 29 patients; 31%) in their analysis. In vitro studies by these investigators also showed that the proliferation of various colorectal cancer cell lines with amplified EGFR was completely inhibited by cetuximab at concentrations that did not affect proliferation of cells with unamplified EGFR (26). Logistic regression analysis indicated that the odds ratio for response to panitumumab was 5.62 (95% CI = 1.51 to 21.0) for increased vs normal mean *EGFR* gene copy number (94).

Overexpression of Other EGFR Ligands. Elevated gene expression of alternative EGFR ligands, such as epiregulin and amphiregulin, may promote tumor growth and survival via an autocrine loop (59,98). Expression of high levels of mRNA for either epiregulin or amphiregulin has been associated with sensitivity to cetuximab monotherapy (59,98). Comparison of clinical outcomes for patients with high and low levels of these ligands showed a statistically significantly improved disease control rate ($P < .001$) and longer progression-free interval among patients with high expression of epiregulin (median = 103.5 vs 57 days, $P < .001$; HR = 0.47 [95% CI = 0.24 to 0.64]) or amphiregulin (median = 115.5 vs 57 days, $P < .001$; HR = 0.44 [95% CI = 0.21 to 0.57]) (59). The exclusive use of either *KRAS* status or amphiregulin or epiregulin gene expression profiles (59) does not result in the selection of identical patient populations who are likely to benefit from treatment with cetuximab: Among patients with wild-type *KRAS*, patients whose tumors expressed high levels of amphiregulin or epiregulin were likely to experience disease control, whereas patients whose tumors expressed low levels of these genes were not, thus providing important complementary information to *KRAS* status (99). Increased gene copy number of HER2 (the preferred heterodimer of EGFR) was linked to a statistically significantly shorter overall survival ($P = .03$), with a trend toward a shorter time to progression ($P = .09$), in 85 patients receiving cetuximab with or without chemotherapy (100).

Other Potential Biomarkers

Markers of angiogenesis and cell cycle regulation appear to be promising areas for further research. Angiogenesis is a prerequisite for growth and progression of tumors (101,102). Although driven by separate mechanisms, EGFR and the key angiogenic factor VEGF-1 share common downstream pathways (Figure 1). Findings of preclinical studies (conducted in human tumor cell lines xenografted into murine models that evaluated the combined pharmacological targeting of EGFR-dependent and VEGF-dependent pathways) indicate direct or indirect angiogenic effects of EGFR signaling (103–106). Furthermore, expression of VEGF-1 or its receptor has been linked to resistance to EGFR-targeted agents in various cancer cell lines (107) and in patients with metastatic colorectal cancer (108). Markers that have been positively linked to outcome in patients with metastatic colorectal cancer who were treated with EGFR-targeted monoclonal antibodies include expression or gene polymorphisms of cyclooxygenase-2 (108,109), interleukin-8 (108,109), and the cell cycle regulator cyclin D1 (93,108,109). For instance, Vallböhmer et al. (108) concluded that a combination of low gene expression levels of cyclooxygenase-2, EGFR, and interleukin-8 was statistically significantly related to the overall survival of patients who were treated with cetuximab monotherapy (13.5 vs 2.3 months in those with high gene expression levels of these three genes; $P = .028$). Feedback mechanisms and complex cellular circuits further link expression of VEGF, interleukin-8, and cyclooxygenase-2 to the oncogenic activation of *KRAS* and *BRAF* genes. For example, expression of cyclooxygenase-2, an upstream regulator of EGFR activity, is driven via the EGFR cascade (Figure 1), and in particular oncogenic *KRAS* has been shown to induce cyclooxygenase-2 expression (110,111). In addition, increased expression of cyclooxygenase-2 may result in increased production of prostaglandin E2, which in turn can transactivate EGFR (112). Expression of the transcription factor nuclear factor κB has also been linked with resistance to cetuximab (113).

Polymorphisms in fragment c gamma receptors, surface receptors for immunoglobulin G located on immune effector cells (such as natural killer lymphocytes and macrophages), are also of interest as potential markers of response to EGFR-targeted monoclonal antibodies, although data are conflicting at present (109,114–116). Fragment c gamma receptors are thought to play a role in antibody-dependent cell-mediated cytotoxicity, which has been postulated as an additional mechanism of action for the IgG1 type of monoclonal antibodies, such as cetuximab, rituximab, and trastuzumab (117). Although it was initially believed that panitumumab, as an IgG2 monoclonal antibody, would not elicit antibody-dependent cell-mediated cytotoxicity, this phenomenon has recently been demonstrated in squamous cell head and neck carcinomas in vitro at concentrations that are analogous to therapeutic doses (117).

Molecular brakes that protect against inappropriate oncogene activation (such as *TP53*) may also be candidate biomarkers of sensitivity to anti-EGFR therapy, given that their inactivation may be required for tumor progression. Indeed, Oden-Gangloff et al. (118) suggest that *TP53* mutations may be predictive of increased likelihood of response to cetuximab treatment, particularly in patients with wild-type *KRAS* status (118).

Early Response Evaluation

The characteristic "acneiform" skin rash observed in most patients who are treated with EGFR inhibitors has been studied as a potential marker of efficacy. This adverse effect is usually apparent after approximately 1 week of treatment and reaches maximum severity after 2–3 weeks. As with the tyrosine kinase inhibitor erlotinib

in patients with lung cancer (24,119), skin toxicity has been consistently linked with higher response rates and longer survival among patients with metastatic colorectal cancer who have been treated with panitumumab (13,120) or cetuximab (7,11,12), whereas patients without rash appear to have a poor outcome. Figure 4 illustrates the relationship between survival and worst grade of rash among patients treated with panitumumab (13) or cetuximab (12) monotherapy in two phase III studies. A landmark analysis (including only patients who were progression free for ≥28 days to allow time for onset) of progression-free interval data from the panitumumab study (n = 231) showed a statistically significant benefit for patients with grade 2–4 skin toxicity compared with those with grade 1 skin toxicity (HR = 0.62, 95% CI = 0.44 to 0.88) (13).

Rash might indicate receptor saturation, and "dose-to-rash" strategies are being studied with the aim of optimizing response to EGFR-targeted treatment. Preliminary data from the phase I–II Evaluation of Various Erbitux Regimens by means of Skin and Tumour biopsies (ie, EVEREST) study suggest that among patients receiving cetuximab-based treatment, cetuximab dose escalation to 500 mg/m^2 per week may improve response rates in those with no or slight skin reactions (121), but the difference was not statistically significant and results require confirmation in a larger study. Subsequent analysis of results by *KRAS* status showed that cetuximab dose escalation did not increase response in patients with tumors carrying a mutant *KRAS* gene. However, among those with tumors carrying wild-type *KRAS*, this strategy improved the response rate from four (21%) of 19 patients to 13 (46%) of 28 patients (122). It is important to note that the panitumumab regimen of 6 mg/kg every 2 weeks, which is approved for treatment of metastatic colorectal cancer, achieves similar drug exposure to a regimen of 2.5 mg/kg per week, which was studied in early phase trials and was found to be associated with a 100% incidence of rash (123).

There are several limitations to the use of rash as an early physical marker of efficacy. As highlighted by De Roock et al. (57), there are no criteria for toxic effects involving skin that are specifically tailored to the activity of EGFR-targeted treatment. Rash often occurs in patients without apparent benefit from anti-EGFR treatment, and conversely, clinical benefit has also been seen in patients without rash (124). Because EGFR is expressed in the skin, skin rash may indicate local receptor saturation, but other factors, such as their immune status, might alter an individual's susceptibility to rash. An association has been observed between tumor and normal tissue with regard to high-affinity EGFR. This finding might provide an explanation for the link observed between skin toxicity and clinical outcome of patients treated with EGFR-targeted treatment (81). Moreover, the relationship between rash and clinical benefit is inconsistent for the tyrosine kinase inhibitor gefitinib (124) and, intriguingly, rash is not observed in patients treated with the humanized anti-EGFR monoclonal antibody nimotuzumab (125,126), although it should be noted that efficacy data for this drug are currently limited.

Anti-EGFR monoclonal antibody treatment may compromise renal magnesium retention capacity, leading to hypomagnesemia in some patients with colorectal cancer (127). Vincenzi et al. (128) recently suggested that reduction in serum magnesium levels might potentially provide an early marker of efficacy of combined

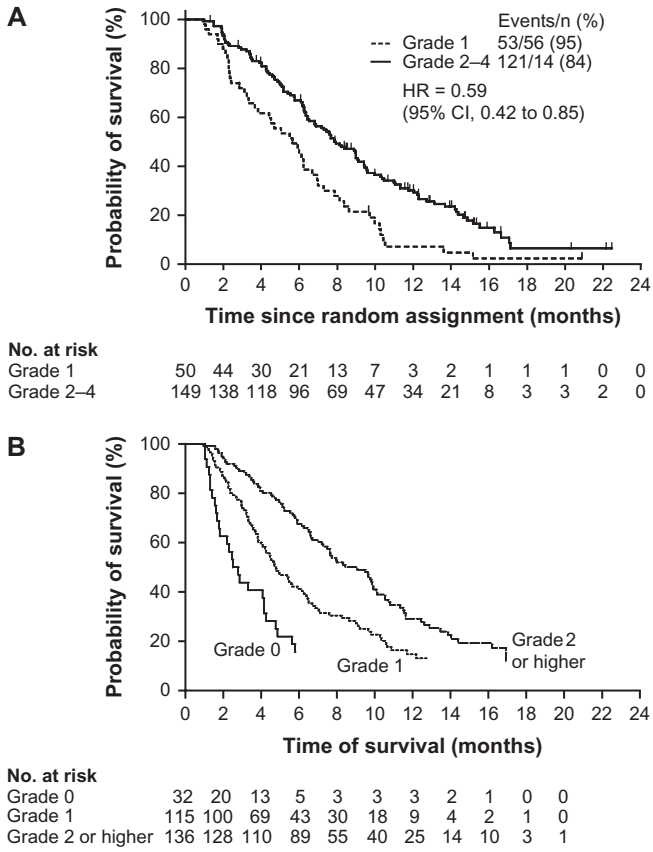

Figure 4. Probability of survival by worst grade of skin toxicity in patients with metastatic colorectal cancer who were treated with EGFR-targeted monoclonal antibodies in two randomized phase III studies. **A**) Patients treated with panitumumab. Data are from a landmark analysis that was limited to patients with progression-free interval of at least 28 days (13) (with permission from the American Society of Clinical Oncology. **B**) Patients treated with cetuximab (12). Reproduced with permission from the *New England Journal of Medicine*. Copyright 2007 Massachusetts Medical Society. All rights reserved. CI = confidence interval; HR = hazard ratio.

treatment with cetuximab and irinotecan. It has also been suggested that treatment with cetuximab may induce a sudden and lasting modulation of circulating VEGF levels (129), although the association between this finding and clinical efficacy was not reported.

Discussion and Future Perspectives

Although the link between clinical benefit and overexpression of the molecular target is clear for trastuzumab and imatinib and their respective targets (HER2 and the BCR-ABL tyrosine kinase), experience has shown that positive expression of EGFR as shown by immunostaining is not predictive of response to EGFR inhibitors. It is now clear that tumor growth can be driven by constitutive activation of signaling pathways downstream of the EGFR, such as the RAS–MAPK–PI3K pathway. Oncogenic activation of components in these pathways can bypass the EGFR-driven signaling cascade and impair the clinical efficacy of anti-EGFR monoclonal antibodies. Such activation can occur via mutations in oncogenes such as *KRAS* or *BRAF* on one side of the EGFR-mediated pathway or by *PIK3CA* mutation or loss of tumor suppressor genes

Table 4. Summary of potential predictive molecular biomarkers for response to the epidermal growth factor receptor (EGFR)–targeted monoclonal antibodies cetuximab and panitumumab in metastatic colorectal cancer*

Relationship to response	Biomarker	First author (reference)
Predicts lack of response and now incorporated into clinical practice†	*KRAS* mutation	Amado (27); Bokemeyer (28); Van Cutsem (29); Table 2, this article; (20,130,131)
Very likely to predict lack of response	Mutation or lack of expression of PTEN; mutation of *BRAF* or *PIK3CA*	Frattini (58); Perrone (38); Benvenuti (33); Di Nicolantonio (40); Sartore-Bianchi (72); Di Nicolantonio (132)
May predict lack of response‡	Increased HER2 gene copy number	Finocchiaro (100)
May predict increased likelihood of response	Increased *EGFR* gene copy number§	Table 3, this article
	Increased EGFR phosphorylation‡	Personeni (81)
	Overexpression of alternative EGFR ligands (amphiregulin and/or epiregulin)‡	Khambata-Ford (59)
	pAKt overexpression‡	Razis (133)
Other potential markers	Markers of angiogenesis and cell cycle regulation; transcription factors (VEGF, IL-8, COX-2, cyclin D, NFκB)‡	Vallböhmer (108); Nagashima (109); Zhang (92)

* Data are based on analysis of tumor tissue from patients participating in clinical trials. COX-2 = cyclooxygenase-2; HER2 = human epidermal growth factor-2; IL-8 = interleukin-8; NFκB = Nuclear factor kappa B; pAkt = phosphorylated Akt; VEGF = vascular endothelial growth factor.

† Based on data from a study that prospectively defined biomarker analysis and included a large number of patients (13).

‡ Limited preliminary data.

§ Data need to be confirmed in large patient datasets, preferably with prospective study design.

such as *PTEN* on the opposite side of the cascade (72) (Figure 1). These findings may provide some explanation for the rather modest objective response rates that have been achieved with clinical trials of EGFR inhibitors to date, as well as the disparities observed between clinical and preclinical findings. It should also be noted that preclinical models are based on particular tumor subtypes that may not be representative of most tumors encountered in clinical practice.

Table 4 summarizes potential biomarkers that may be related to primary response to the anti-EGFR monoclonal antibodies, panitumumab and cetuximab. Overall, presently available data reviewed in this article provide convincing evidence that activating mutations of *KRAS*, which are present in a substantial proportion of patients with metastatic colorectal cancer, predict lack of response to anti-EGFR monoclonal antibody treatment (27–29, 33,34,55,60). This finding is consistent with observations from use of EGFR tyrosine kinase inhibitors in the treatment of non–small cell lung cancer, although *KRAS* mutations are less common in lung tumors (66,67).

KRAS testing is now being integrated into clinical practice. The European Medicines Agency's conditional approval of panitumumab monotherapy in the setting of chemorefractory metastatic colorectal cancer specified wild-type *KRAS* as a selection marker (134). Current data indicate that objective response rates of up to 22% can be expected in such patients (Table 2). It should be noted that clinical benefit is not confined to objective responders because delaying disease progression can improve clinical symptoms and the patient's quality of life (135). Wild-type *KRAS* was more recently identified as a selection marker for cetuximab monotherapy or combination therapy (136). The European Medicines Agency (20, 130), the US Food and Drug Administration (137), and the American Society of Oncology (131) now recommend determining tumor *KRAS* status before initiating treatment with an anti-EGFR monoclonal antibody and restricting such treatment to patients with tumors bearing wild-type *KRAS*.

Because of the complexity of the EGFR signaling system, it is likely that predictive algorithms will be developed for metastatic colorectal cancer that incorporate several molecular biomarkers. For instance, combining analysis of *KRAS* status with determination of *BRAF* and *PIK3CA* status and PTEN expression may identify additional patients with metastatic colorectal cancer who are unlikely to respond to treatment with an EGFR-targeted monoclonal antibody (133). However, these additional markers (Table 4) require further validation before they can be incorporated into clinical practice.

Tumors with an increased *EGFR* gene copy number as assessed by FISH or chromogenic in situ hybridization may be dependent on the EGFR pathway for their survival and growth. There is evidence that normal diploid *EGFR* gene copy number may predict tumor resistance to EGFR-targeted treatment (Table 3). Again, further research is required to validate this biomarker in larger patient series (97).

There are clearly a number of technical issues to be overcome, particularly standardization of analytical methods and scoring systems. Lack of standardization may well explain some of the discordant results that have been reported. Certainly, some biomarkers may not prove suitable for translation into clinical practice. However, DNA sequencing of formalin-fixed paraffin-embedded tumor samples is a relatively straightforward method for identifying *KRAS* mutations. Because *KRAS* mutation is an early event in colorectal cancer tumorigenesis (42,43), archived primary tumor tissue can be used to identify patients who are unlikely to respond to EGFR-targeted monoclonal antibodies, even after multiple lines of treatment. A *KRAS* testing kit from DxS Ltd can identify the following seven somatic mutations in *KRAS* codons 12 and 13: Gly12Ala, Gly12Arg, Gly12Asp, Gly12Ser, Gly12Cys, Gly12 Val, and Gly13Asp. The polymerase chain reaction–based technique used is highly sensitive but does not detect less frequent changes that can be detected with direct sequencing (eg, Gly13Val, Gly13Ala, and Gly13Cys). Although direct sequencing has the capability to detect all changes at the nucleotide level, it is less

sensitive and may occasionally miss mutations, especially if the fraction of tumor vs normal cells is low (57). Testing will be facilitated by gene panel microarray technology and gene panels that incorporate *KRAS* testing are being evaluated (138).

Further work is also required to explore potential early markers of response (in patients already receiving EGFR-targeted treatment) that can be incorporated into the design of future prospective clinical trials and guide therapeutic decisions regarding continuation of treatment in individual patients. Skin toxicity develops at an early stage in treatment with EGFR inhibitors and has been studied extensively as a potential early marker of response, although further work is required.

Virtually all responding tumors eventually "escape" from EGFR-targeted treatment (ie, develop acquired resistance). In the lung cancer setting, acquired resistance to erlotinib or gefitinib has been attributed to development of a secondary mutation within the *EGFR* catalytic domain (139). Other potential escape mechanisms include activation of alternative signaling pathways contributing to proliferation and survival, such as those involving activation of HER2, HER3, mesenchymal–epithelial transition factor (C-MET), insulin-like growth factor-I receptor, MAPK, and Akt (140). Consequently, identification of the molecular basis of acquired resistance to anti-EGFR monoclonal antibodies in metastatic colorectal cancer should be a priority for future research.

In conclusion, the quest for predictive biomarkers of response to EGFR inhibitor therapy has resulted in a rapidly accumulating body of knowledge that has paved the way for more targeted use of these agents. More rational use of EGFR-targeted agents should provide benefits for patients and health-care providers alike by sparing patients unnecessary treatment and allowing better use of health-care resources. Prospective biomarker-driven studies are now under way, but in the meantime, the identification of wild-type *KRAS* as a selection biomarker for panitumumab or cetuximab therapy represents an important step toward fulfilling the promise of individualized treatment for metastatic colorectal cancer.

References

1. Yarden Y, Sliwkowski MX. Untangling the ErbB signalling network. *Nat Rev Mol Cell Biol.* 2001;2(2):127–137.
2. Scaltriti M, Baselga J. The epidermal growth factor receptor pathway: a model for targeted therapy. *Clin Cancer Res.* 2006;12(18):5268–5272.
3. Harari PM. Epidermal growth factor receptor inhibition strategies in oncology. *Endocr Relat Cancer.* 2004;11(4):689–708.
4. Nicholson RI, Gee JM, Harper ME. EGFR and cancer prognosis. *Eur J Cancer.* 2001;37(suppl 4):S9–S15.
5. Mackenzie MJ, Hirte HW, Glenwood G, et al. A phase II trial of ZD1839 (Iressa) 750 mg per day, an oral epidermal growth factor receptor-tyrosine kinase inhibitor, in patients with metastatic colorectal cancer. *Invest New Drugs.* 2005;23(2):165–170.
6. Santoro A, Comandone A, Rimassa L, et al. A phase II randomized multicenter trial of gefitinib plus FOLFIRI and FOLFIRI alone in patients with metastatic colorectal cancer. *Ann Oncol.* 2008;19(11):1888–1893.
7. 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):337–345.
8. Sobrero AF, 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):2311–2319.
9. Van Cutsem E, Nowacki M, Lang I, et al. Randomized phase III study of irinotecan and 5-FU/FA with or without cetuximab in the first-line treatment of patients with metastatic colorectal cancer (mCRC): the CRYSTAL trial [abstract 4000]. *J Clin Oncol.* 2007;25:(suppl).
10. Bokemeyer C, Bondarenko I, Makhson A, et al. Cetuximab plus 5-FU/FA/oxaliplatin (FOLFOX-4) versus FOLFOX-4 in the first-line treatment of metastatic colorectal cancer (mCRC): OPUS, a randomized phase II study [abstract 4035]. *J Clin Oncol.* 2007;25:(suppl).
11. Saltz LB, Meropol NJ, Loehrer PJ Sr, et al. Phase II trial of cetuximab in patients with refractory colorectal cancer that expresses the epidermal growth factor receptor. *J Clin Oncol.* 2004;22(7):1201–1208.
12. Jonker DJ, O'Callaghan CJ, Karapetis CS, et al. Cetuximab for the treatment of colorectal cancer. *N Engl J Med.* 2007;357(20):2040–2048.
13. 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):1658–1664.
14. Peeters M, Wilson G, Ducreux M, et al. Phase III study (20050181) of panitumumab (pmab) with FOLFIRI versus FOLFIRI alone as second-line treatment (tx) in patients (pts) with metastatic colorectal cancer (mCRC): pooled safety results [abstract 4064]. *J Clin Oncol.* 2008;26:suppl.
15. Siena S, Tabernero J, Burkes RL, et al. Phase III study (PRIME/20050203) of panitumumab (pmab) with FOLFOX compared with FOLFOX alone in patients (pts) with previously untreated metastatic colorectal cancer (mCRC): pooled safety data [abstract 4034]. *J Clin Oncol.* 2008;26:(suppl).
16. 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):672–680.
17. Tol J, Koopman M, Cats A, et al. Chemotherapy, bevacizumab, and cetuximab in metastatic colorectal cancer. *N Engl J Med.* 2009;360(6):563–572.
18. Hecht JR, Patnaik A, Berlin J, et al. Panitumumab monotherapy in patients with previously treated metastatic colorectal cancer. *Cancer.* 2007;110(5):980–988.
19. O'Neil BH, Allen R, Spigel DR, et al. High incidence of cetuximab-related infusion reactions in Tennessee and North Carolina and the association with atopic history. *J Clin Oncol.* 2007;25(24):3644–3648.
20. Anonymous. Summary of product characteristics (Erbitux). http://www.emea.europa.eu/humandocs/PDFs/EPAR/erbitux/H-558-PI-en.pdf. Accessed July 21, 2009.
21. Chung CH, Mirakhur B, Chan E, et al. Cetuximab-induced anaphylaxis and IgE specific for galactose-alpha-1,3-galactose. *N Engl J Med.* 2008;358(11):1109–1117.
22. Chung KY, Shia J, Kemeny NE, 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):1803–1810.
23. Mitchell EP, Hecht JR, Baranda J, et al. Panitumumab activity in metastatic colorectal cancer (mCRC) patients (pts) with low or negative tumor epidermal growth factor receptor (EGFr) levels: an updated analysis [abstract 4082]. *J Clin Oncol.* 2007;25(18)(suppl).
24. Perez-Soler R, Chachoua A, Hammond LA, et al. Determinants of tumor response and survival with erlotinib in patients with non-small-cell lung cancer. *J Clin Oncol.* 2004;22(16):3238–3247.
25. Parra HS, Cavina R, Latteri F, et al. Analysis of epidermal growth factor receptor expression as a predictive factor for response to gefitinib ('Iressa', ZD1839) in non-small-cell lung cancer. *Br J Cancer.* 2004;91(2):208–212.
26. 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):279–286.
27. 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):1626–1634.
28. Bokemeyer C, Bondarenko I, Makhson A, et al. Fluorouracil, leucovorin, and oxaliplatin with and without cetuximab in the first-line treatment of metastatic colorectal cancer. *J Clin Oncol.* 2009;27(5):663–671.
29. Van Cutsem E, Köhne CH, Hitre E, et al. Cetuximab and chemotherapy as initial treatment for metastatic colorectal cancer. *N Engl J Med.* 2009;360(14):1408–1417.

30. McCubrey JA, Steelman LS, Abrams SL, et al. Roles of the RAF/MEK/ERK and PI3K/PTEN/AKT pathways in malignant transformation and drug resistance. *Adv Enzyme Regul.* 2006;46(1):249–279.
31. Bos JL. ras oncogenes in human cancer: a review. *Cancer Res.* 1989;49(17):4682–4689.
32. Barault L, Veyrie N, Jooste V, et al. Mutations in the RAS-MAPK, PI(3)K (phosphatidylinositol-3-OH kinase) signaling network correlate with poor survival in a population-based series of colon cancers. *Int J Cancer.* 2008;122(10):2255–2259.
33. Benvenuti S, Sartore-Bianchi A, Di Nicolantonio F, et al. Oncogenic activation of the RAS/RAF signaling pathway impairs the response of metastatic colorectal cancers to anti-epidermal growth factor receptor antibody therapies. *Cancer Res.* 2007;67(6):2643–2648.
34. Lievre A, Bachet JB, Le Corre D, et al. KRAS mutation status is predictive of response to cetuximab therapy in colorectal cancer. *Cancer Res.* 2006;66(8):3992–3995.
35. Frattini M, Signoroni S, Pilotti S, et al. Phosphatase protein homologue to tensin expression and phosphatidylinositol-3 phosphate kinase mutations in colorectal cancer. *Cancer Res.* 2005;65(23):11227.
36. Freeman DJ, Juan T, Reiner M, et al. Association of K-ras mutational status and clinical outcomes in patients with metastatic colorectal cancer receiving panitumumab alone. *Clin Colorectal Cancer.* 2008;7(3):184–190.
37. Velho S, Oliveira C, Ferreira A, et al. The prevalence of PIK3CA mutations in gastric and colon cancer. *Eur J Cancer.* 2005;41(11):1649–1654.
38. Perrone F, Lampis A, Orsenigo M, et al. PI3KCA/PTEN deregulation contributes to impaired responses to cetuximab in metastatic colorectal cancer patients. *Ann Oncol.* 2009;20(1):84–90.
39. Rajagopalan H, Bardelli A, Lengauer C, et al. Tumorigenesis: RAF/RAS oncogenes and mismatch-repair status. *Nature.* 2002;418(6901):934.
40. 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):5705–5712.
41. Tol J, Nagtegaal I, Punt CJA. BRAF mutation in metastatic colorectal cancer [letter to editor]. *N Engl J Med.* 2009;361(1):98–99.
42. Fearon ER, Vogelstein B. A genetic model for colorectal tumorigenesis. *Cell.* 1990;61(5):759–767.
43. Andreyev HJ, Norman AR, Cunningham D, et al. Kirsten ras mutations in patients with colorectal cancer: the 'RASCAL II' study. *Br J Cancer.* 2001;85(5):692–696.
44. Zauber P, Sabbath-Solitare M, Marotta SP, Bishop DT. Molecular changes in the Ki-ras and APC genes in primary colorectal carcinoma and synchronous metastases compared with the findings in accompanying adenomas. *Mol Pathol.* 2003;56(3):137–140.
45. Artale S, Sartore-Bianchi A, Veronese S, et al. Mutations of KRAS and BRAF in primary and matched metastatic sites of colorectal cancer. *J Clin Oncol.* 2008;26(25):4217–4219.
46. Etienne-Grimaldi MC, Formento JL, Francoual M, et al. K-Ras mutations and treatment outcome in colorectal cancer patients receiving exclusive fluoropyrimidine therapy. *Clin Cancer Res.* 2008;14(15):4830–4835.
47. Albanese I, Scibetta AG, Migliavacca M, et al. Heterogeneity within and between primary colorectal carcinomas and matched metastases as revealed by analysis of Ki-ras and p53 mutations. *Biochem Biophys Res Commun.* 2004;325(3):784–791.
48. Oudejans JJ, Slebos RJ, Zoetmulder FA, Mooi WJ, Rodenhuis S. Differential activation of ras genes by point mutation in human colon cancer with metastases to either lung or liver. *Int J Cancer.* 1991;49(6):875–879.
49. Esteller M, Gonzalez S, Risques RA, et al. K-ras and p16 aberrations confer poor prognosis in human colorectal cancer. *J Clin Oncol.* 2001;19(2):299–304.
50. Bazan V, Migliavacca M, Zanna I, et al. Specific codon 13 K-ras mutations are predictive of clinical outcome in colorectal cancer patients, whereas codon 12 K-ras mutations are associated with mucinous histotype. *Ann Oncol.* 2002;13(9):1438–1446.
51. Markowitz S, Hines JD, Lutterbaugh J, et al. Mutant K-ras oncogenes in colon cancers do not predict patient's chemotherapy response or survival. *Clin Cancer Res.* 1995;1(4):441–445.
52. Dix BR, Robbins P, Soong R, et al. The common molecular genetic alterations in Dukes' B and C colorectal carcinomas are not short-term prognostic indicators of survival. *Int J Cancer.* 1994;59(6):747–751.
53. Morrin M, Kelly M, Barrett N, Delaney P. Mutations of Ki-ras and p53 genes in colorectal cancer and their prognostic significance. *Gut.* 1994;35(11):1627–1631.
54. Roth A, Tejpar S, Yan P, et al. Correlation of molecular markers in colon cancer with stage-specific prognosis: results of the translational study on the PETACC 3—EORTC 40993-SAKK 60-00 trial. Presented at: American Society of Clinical Oncology 2009 Gastrointestinal Cancers Symposium, Jan 15–17; San Francisco, CA [abstract 288]. http://www.asco.org/ASCO/Abstracts+%26+Virtual+Meeting/Abstracts?&vmview=abst_detail_view&confID=63&abstractID=10437. Accessed July 21.
55. Karapetis CS, Khambata-Ford S, Jonker DJ, et al. K-ras mutations and benefit from cetuximab in advanced colorectal cancer. *N Engl J Med.* 2008;359(17):1757–1765.
56. Di Fiore F, Blanchard F, Charbonnier F, et al. Clinical relevance of KRAS mutation detection in metastatic colorectal cancer treated by cetuximab plus chemotherapy. *Br J Cancer.* 2007;96(8):1166–1169.
57. De Roock W, Piessevaux H, De Schutter J, et al. KRAS wild-type state predicts survival and is associated to early radiological response in metastatic colorectal cancer treated with cetuximab. *Ann Oncol.* 2008;19(3):508–515.
58. 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):1139–1145.
59. Khambata-Ford S, Garrett CR, Meropol NJ, et al. Expression of epiregulin and amphiregulin and K-ras mutation status predict disease control in metastatic colorectal cancer patients treated with cetuximab. *J Clin Oncol.* 2007;25(22):3230–3237.
60. Lievre A, Bachet JB, Boige V, et al. KRAS mutations as an independent prognostic factor in patients with advanced colorectal cancer treated with cetuximab. *J Clin Oncol.* 2008;26(3):374–379.
61. Amgen Inc. Data on file. Thousand Oaks, CA. 2008.
62. Moore MJ, Goldstein D, Hamm J, et al. Erlotinib plus gemcitabine compared with gemcitabine alone in patients with advanced pancreatic cancer: a phase III trial of the National Cancer Institute of Canada Clinical Trials Group. *J Clin Oncol.* 2007;25(15):1960–1966.
63. Philip PA, Benedetti J, Fenoglio-Preiser C, et al. Phase III study of gemcitabine [G] plus cetuximab [C] versus gemcitabine in patients [pts] with locally advanced or metastatic pancreatic adenocarcinoma [PC]: SWOG S0205 study [2007 ASCO Meeting abstracts]. *J Clin Oncol.* 2007;25(18)(suppl):LBA4509.
64. Sempere LF, Korc M. Shining the spotlight on shed KRAS in pancreatic cancer. *Cancer Biol Ther.* 2008;7(3):361–363.
65. Pao W, Wang TY, Riely GJ, et al. KRAS mutations and primary resistance of lung adenocarcinomas to gefitinib or erlotinib. *PLoS Med.* 2005;2(1):e17.
66. Massarelli E, Varella-Garcia M, Tang X, et al. KRAS mutation is an important predictor of resistance to therapy with epidermal growth factor receptor tyrosine kinase inhibitors in non-small-cell lung cancer. *Clin Cancer Res.* 2007;13(10):2890–2896.
67. Eberhard DA, Johnson BE, Amler LC, et al. Mutations in the epidermal growth factor receptor and in KRAS are predictive and prognostic indicators in patients with non-small-cell lung cancer treated with chemotherapy alone and in combination with erlotinib. *J Clin Oncol.* 2005;23(25):5900–5909.
68. Bianco R, Shin I, Ritter CA, et al. Loss of PTEN/MMAC1/TEP in EGF receptor-expressing tumor cells counteracts the antitumor action of EGFR tyrosine kinase inhibitors. *Oncogene.* 2003;22(18):2812–2822.
69. Mellinghoff IK, Wang MY, Vivanco I, et al. Molecular determinants of the response of glioblastomas to EGFR kinase inhibitors. *N Engl J Med.* 2005;353(19):2012–2024.
70. Nagata Y, Lan KH, Zhou X, et al. PTEN activation contributes to tumor inhibition by trastuzumab, and loss of PTEN predicts trastuzumab resistance in patients. *Cancer Cell.* 2004;6(2):117–127.
71. Jhawer M, Goel S, Wilson AJ, et al. PIK3CA mutation/PTEN expression status predicts response of colon cancer cells to the epidermal growth factor receptor inhibitor cetuximab. *Cancer Res.* 2008;68(6):1953–1961.

72. 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):1851–1857.
73. 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):3184–3188.
74. Razis E, Briasoulis E, Vrettou E, et al. Potential value of PTEN in predicting cetuximab response in colorectal cancer. An exploratory study. *BMC Cancer.* 2008;8:234.
75. Yuan TL. Cantley LC. PI3K pathway alterations in cancer: variations on a theme. *Oncogene.* 2008;27(41):5497–5510.
76. Bianco R, Garofalo S, Rosa R, et al. Inhibition of mTOR pathway by everolimus cooperates with EGFR inhibitors in human tumours sensitive and resistant to anti-EGFR drugs. *Br J Cancer.* 2008;98(5): 923–930.
77. Benvenuti S, Frattini M, Arena S, et al. PIK3CA cancer mutations display gender and tissue specificity patterns. *Hum Mutat.* 2008;29(2):284–288.
78. Shia J, Klimstra DS, Li AR, et al. Epidermal growth factor receptor expression and gene amplification in colorectal carcinoma: an immunohistochemical and chromogenic in situ hybridization study. *Mod Pathol.* 2005;18(10): 1350–1356.
79. Scartozzi M, Bearzi I, Berardi R, et al. Epidermal growth factor receptor (EGFR) status in primary colorectal tumors does not correlate with EGFR expression in related metastatic sites: implications for treatment with EGFR-targeted monoclonal antibodies. *J Clin Oncol.* 2004;22(23): 4772–4778.
80. Francoual M, Etienne-Grimaldi MC, Formento JL, et al. EGFR in colorectal cancer: more than a simple receptor. *Ann Oncol.* 2006;17(6): 962–967.
81. Personeni N, Hendlisz A, Gallez J, et al. Correlation between the response to cetuximab alone or in combination with irinotecan and the activated/phosphorylated epidermal growth factor receptor in metastatic colorectal cancer. *Semin Oncol.* 2005;32(6)(suppl 9):S59–S62.
82. Taron M, Ichinose Y, Rosell R, et al. Activating mutations in the tyrosine kinase domain of the epidermal growth factor receptor are associated with improved survival in gefitinib-treated chemorefractory lung adenocarcinomas. *Clin Cancer Res.* 2005;11(16):5878–5885.
83. Sequist LV, Joshi VA, Janne PA, et al. Response to treatment and survival of patients with non-small cell lung cancer undergoing somatic EGFR mutation testing. *Oncologist.* 2007;12(1):90–98.
84. Barber TD, Vogelstein B, Kinzler KW, Velculescu VE. Somatic mutations of EGFR in colorectal cancers and glioblastomas. *N Engl J Med.* 2004;351(27):2883.
85. Lenz HJ, Van Cutsem E, Khambata-Ford S, et al. Multicenter phase II and translational study of cetuximab in metastatic colorectal carcinoma refractory to irinotecan, oxaliplatin, and fluoropyrimidines. *J Clin Oncol.* 2006;24(30):4914–4921.
86. Moroni M, Sartore-Bianchi A, Benvenuti S, et al. Somatic mutation of EGFR catalytic domain and treatment with gefitinib in colorectal cancer. *Ann Oncol.* 2005;16(11):1848–1849.
87. Press OA, Zhang W, Gordon MA, et al. Gender-related survival differences associated with EGFR polymorphisms in metastatic colon cancer. *Cancer Res.* 2008;68(8):3037–3042.
88. Goncalves A, Esteyries S, Taylor-Smedra B, et al. A polymorphism of EGFR extracellular domain is associated with progression free-survival in metastatic colorectal cancer patients receiving cetuximab-based treatment. *BMC Cancer.* 2008;8:169.
89. Graziano F, Ruzzo A, Loupakis F, et al. Pharmacogenetic profiling for cetuximab plus irinotecan therapy in patients with refractory advanced colorectal cancer. *J Clin Oncol.* 2008;26(9):1427–1434.
90. Wang WS, Chen PM, Chiou TJ, et al. Epidermal growth factor receptor R497K polymorphism is a favorable prognostic factor for patients with colorectal carcinoma. *Clin Cancer Res.* 2007;13(12):3597–3604.
91. Garm Spindler KL, Pallisgaard N, Rasmussen AA, et al. The importance of KRAS mutations and EGF61A>G polymorphism to the effect of cetuximab and irinotecan in metastatic colorectal cancer. *Ann Oncol.* 2009;20(5): 879–884.
92. Zhang W, Gordon M, Press OA, et al. Cyclin D1 and epidermal growth factor polymorphisms associated with survival in patients with advanced colorectal cancer treated with cetuximab. *Pharmacogenet Genomics.* 2006;16(7): 475–483.
93. Ooi A, Takehana T, Li X, et al. Protein overexpression and gene amplification of HER-2 and EGFR in colorectal cancers: an immunohistochemical and fluorescent in situ hybridization study. *Mod Pathol.* 2004;17(8): 895–904.
94. 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):3238–3245.
95. Cappuzzo F, Finocchiaro G, Rossi E, et al. EGFR FISH assay predicts for response to cetuximab in chemotherapy refractory colorectal cancer patients. *Ann Oncol.* 2008;19(4):717–723.
96. Personeni N, Fieuws S, Piessevaux H, et al. Clinical usefulness of EGFR gene copy number as a predictive marker in colorectal cancer patients treated with cetuximab: a fluorescent in situ hybridization study. *Clin Cancer Res.* 2008;14(18):5869–5876.
97. Moroni M, Sartore-Bianchi A, Veronese S, Siena S. EGFR FISH in colorectal cancer: what is the current reality? *Lancet Oncol.* 2008;9(5): 402–403.
98. Jacobs B, Biesmans B, De Roock W, De Schutter J, Van Cutsem E, Tejpar E. Epiregulin and amphiregulin expression defines a subset of colorectal tumors sensitive to EGFR inhibition. In: Proceedings of the 100th Annual Meeting of the American Association for Cancer Research; April 18–22, 2009; Denver, CO: AACR; 2009. Abstract 1346.
99. Harbison CT, Mauro DJ, Clark EA, Khambata Ford S. In reply. *J Clin Oncol.* 2008;26(13):2230–2231.
100. Finocchiaro G, Cappuzzo F, Janne PA, et al. EGFR, HER2 and Kras as predictive factors for cetuximab sensitivity in colorectal cancer [abstract 4021]. *J Clin Oncol.* 2007;25(suppl).
101. Folkman J. Angiogenesis in cancer, vascular, rheumatoid and other disease. *Nat Med.* 1995;1(1):27–31.
102. Ellis LM. Angiogenesis and its role in colorectal tumor and metastasis formation. *Semin Oncol.* 2004;31(6)(suppl 17):3–9.
103. Ellis LM. Epidermal growth factor receptor in tumor angiogenesis. *Hematol Oncol Clin North Am.* 2004;18(5):1007–1021.
104. Ciardiello F, Troiani T, Bianco R, et al. Interaction between the epidermal growth factor receptor (EGFR) and the vascular endothelial growth factor (VEGF) pathways: a rational approach for multi-target anticancer therapy. *Ann Oncol.* 2006;17(suppl 7):vii109–vii114.
105. Ciardiello F, Bianco R, Caputo R, et al. Antitumor activity of ZD6474, a vascular endothelial growth factor receptor tyrosine kinase inhibitor, in human cancer cells with acquired resistance to antiepidermal growth factor receptor therapy. *Clin Cancer Res.* 2004;10(2):784–793.
106. Tortora G, Ciardiello F, Gasparini G. Combined targeting of EGFR-dependent and VEGF-dependent pathways: rationale, preclinical studies and clinical applications. *Nat Clin Pract Oncol.* 2008;5(9):521–530.
107. Bianco R, Rosa R, Damiano V, et al. Vascular endothelial growth factor receptor-1 contributes to resistance to anti-epidermal growth factor receptor drugs in human cancer cells. *Clin Cancer Res.* 2008;14(16): 5069–5080.
108. Vallböhmer D, Zhang W, Gordon M, et al. Molecular determinants of cetuximab efficacy. *J Clin Oncol.* 2005;23(15):3536–3544.
109. Nagashima F, Zhang W, Gordon M, et al. EGFR, Cox-2, and EGF polymorphisms associated with progression-free survival of EGFR-expressing metastatic colorectal cancer patients treated with single-agent cetuximab (IMCL-0144) [abstract 4129]. *J Clin Oncol.* 2007;25(suppl).
110. Wang XQ, Li H, Van Putten V, Winn RA, Heasley LE, Nemenoff RA. Oncogenic K-Ras regulates proliferation and cell junctions in lung epithelial cells through induction of cyclooxygenase-2 and activation of metalloproteinase-9. *Mol Biol Cell.* 2009;20(3):791–800.
111. Smakman N, Kranenburg O, Vogten JM, Bloemendaal AL, van Diest P, Borel Rinkes IH. Cyclooxygenase-2 is a target of KRASD12, which facilitates the outgrowth of murine C26 colorectal liver metastases. *Clin Cancer Res.* 2005;11(1):41–48.
112. Pai R, Soreghan B, Szabo IL, Pavelka M, Baatar D, Tarnawski AS. Prostaglandin E2 transactivates EGF receptor: a novel mechanism for

promoting colon cancer growth and gastrointestinal hypertrophy. *Nat Med.* 2002;8(3):289–293.
113. Scartozzi M, Bearzi I, Pierantoni C, et al. Nuclear factor-kB tumor expression predicts response and survival in irinotecan-refractory metastatic colorectal cancer treated with cetuximab-irinotecan therapy. *J Clin Oncol.* 2007;25(25):3930–3935.
114. Zhang W, Gordon M, Schultheis AM, et al. FCGR2A and FCGR3A polymorphisms associated with clinical outcome of epidermal growth factor receptor expressing metastatic colorectal cancer patients treated with single-agent cetuximab. *J Clin Oncol.* 2007;25(24):3712–3718.
115. Bibeau F, Lopez-Crapez E, Di Fiore F, et al. Impact of Fc{gamma}RIIa-Fc{gamma}RIIIa polymorphisms and KRAS mutations on the clinical outcome of patients with metastatic colorectal cancer treated with cetuximab plus irinotecan. *J Clin Oncol.* 2009;27(7):1122–1129.
116. Negri F, Musolino A, Naldi N, et al. Immunoglobulin G fragment C receptor polymorphisms and clinical outcome of EGFR-expressing metastatic colorectal cancer patients treated with cetuximab-based therapy. Abstract presented at: 14th ECCO; September 23–27, 2007; Barcelona, Spain. *Eur J Cancer*. 2007;5(4 suppl):96.
117. Lopez-Albaitero A, Ferris RL. Immune activation by epidermal growth factor receptor specific monoclonal antibody therapy for head and neck cancer. *Arch Otolaryngol Head Neck Surg.* 2007;133(12):1277–1281.
118. Oden-Gangloff A, Di Fiore F, Bibeau F, et al. *TP53* mutations predict disease control in metastatic colorectal cancer treated with cetuximab-based chemotherapy. *Br J Cancer.* 2009;100(8):1330–1335.
119. Herbst RS, Prager D, Hermann R, et al. TRIBUTE: a phase III trial of erlotinib hydrochloride (OSI-774) combined with carboplatin and paclitaxel chemotherapy in advanced non-small-cell lung cancer. *J Clin Oncol.* 2005;23(25):5892–5899.
120. Berlin J, Van Cutsem E, Peeters M, et al. Predictive value of skin toxicity severity for response to panitumumab in patients with metastatic colorectal cancer (mCRC): a pooled analysis of five clinical trials [abstract 4134]. *J Clin Oncol.* 2007;25(suppl).
121. Tejpar S, Peeters M, Humblet Y, et al. Phase I/II study of cetuximab dose-escalation in patients with metastatic colorectal cancer (mCRC) with no or slight skin reactions on cetuximab standard dose treatment (EVEREST): pharmacokinetic (PK), pharmacodynamic (PD) and efficacy data [abstract 4037]. *J Clin Oncol.* 2007;25(suppl).
122. Tejpar S, Peeters M, Humblet Y, et al. Relationship of efficacy with KRAS status (wild type versus mutant) in patients with irinotecan-refractory metastatic colorectal cancer (mCRC), treated with irinotecan (q2w) and escalating doses of cetuximab (q1w): the EVEREST experience (preliminary data) [abstract 4001]. *J Clin Oncol.* 2008;26(suppl).
123. 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):3003–3015.
124. Perez-Soler R, Saltz L. Cutaneous adverse effects with HER1/EGFR-targeted agents: is there a silver lining? *J Clin Oncol.* 2005;23(22):5235–5246.
125. Allan DGP. Nimotuzumab: evidence of clinical benefit without rash. *Oncologist.* 2005;10(9):760–761.
126. Crombet T, Osorio M, Cruz T, et al. Use of the humanized anti-epidermal growth factor receptor monoclonal antibody h-R3 in combination with radiotherapy in the treatment of locally advanced head and neck cancer patients. *J Clin Oncol.* 2004;22(9):1646–1654.
127. Tejpar S, Piessevaux H, Claes K, et al. Magnesium wasting associated with epidermal-growth factor receptor-targeting antibodies in colorectal cancer: a prospective study. *Lancet Oncol.* 2007;85(5):387–394.
128. Vincenzi B, Santini D, Galluzzo S, et al. Early magnesium reduction in advanced colorectal cancer patients treated with cetuximab plus irinotecan as predictive factor of efficacy and outcome. *Clin Cancer Res.* 2008;14(13):4219–4224.
129. Vincenzi B, Santini D, Russo A, et al. Angiogenesis modifications related with cetuximab plus irinotecan as anticancer treatment in advanced colorectal cancer patients. *Ann Oncol.* 2006;17(5):835–841.
130. Anonymous. Summary of product characteristics (Vectibix). http://www.emea.europa.eu/humandocs/PDFs/EPAR/vectibix/H-741-PI-en.pdf. Accessed July 21, 2009.
131. Allegra CJ, Jessup JM, Somerfield MR, et al. American Society of Clinical Oncology provisional clinical opinion: testing for KRAS gene mutations in patients with metastatic colorectal carcinoma to predict response to anti-epidermal growth factor receptor monoclonal antibody therapy. *J Clin Oncol.* 2009;27(12):2091–2096.
132. Di Nicolantonio F, Sartore-Bianchi A, Molinari F, et al. BRAF, PIK3CA, and KRAS mutations and loss of PTEN expression impair response to EGFR-targeted therapies in metastatic colorectal cancer. In: Proceedings of the 100th Annual Meeting of the American Association for Cancer Research; April 18–22, 2009; Denver, CO: AACR; 2009. Abstract LB-93.
133. Razis E, Briasoulis E, Kostopoulos I, et al. Predictive markers for the treatment of colorectal cancer with cetuximab [O Meeting abstract 13500]. *J Clin Oncol*. 2006;24(18)(suppl).
134. European Medicines Agency. Committee for Medicinal Products for Human Use December 2007 Plenary Meeting monthly report. http://www.emea.europa.eu/pdfs/human/press/pr/58563707en.pdf. Accessed July 21, 2009.
135. Siena S, Peeters M, Van Cutsem E, et al. Association of progression-free survival with patient-reported outcomes and survival: results from a randomised phase 3 trial of panitumumab. *Br J Cancer.* 2007;97(11):1469–1474.
136. European Medicines Agency. Committee for Medicinal Products for Human Use May 2008 Plenary Meeting monthly report. http://www.emea.europa.eu/pdfs/human/press/pr/27923508en.pdf. Accessed July 21, 2009.
137. Anonymous. FDA adds KRAS testing info to Vectibix, Erbitux Labels (Press Release, Genome Web News). http://www.genomeweb.com/dxpgx/fda-adds-kras-testing-info-vectibix-erbitux-labels. Accessed July 21, 2009.
138. Baker J, Dutta D, Watson D, et al. Evaluation of tumor gene expression and K-Ras mutations in FFPE tumor tissue as predictors of response to cetuximab in metastatic colorectal cancer [abstract 3512]. *J Clin Oncol.* 2008;26(suppl).
139. Sharma SV, Bell DW, Settleman J, Haber DA. Epidermal growth factor receptor mutations in lung cancer. *Nat Rev Cancer.* 2007;7(3):169–181.
140. Wheeler DL, Huang S, Kruser TJ, et al. Mechanisms of acquired resistance to cetuximab: role of HER (ErbB) family members. *Oncogene.* 2008;27(28):3944–3956.

Funding

Funding for the writing of this manuscript was provided by Amgen (Europe) GmbH, Zug, Switzerland. This work was partly supported by grants from Oncologia Ca' Granda Onlus Fondazione, Associazione Italiana Ricerca Cancro, Italian Ministry of Health, Regione Piemonte, EU FP6 MSCs contract 037297, EU FP7 Marie Curie, contract n 218071, CRT Progetto Alfieri. Amgen (Europe) GmbH had the opportunity to review the manuscript for accuracy.

Notes

J. Balfour was an employee of Amgen at the time that writing of this article commenced. She is currently a self-employed medical writer.

The authors had full responsibility for identification of articles, analysis and interpretation of the data, writing of the manuscript, and the decision to submit the manuscript for publication.

S. Siena, A. Sartore-Bianchi, A. Bardelli, and F. Di Nicolantonio have no financial interests, arrangements, or connections to declare.

Manuscript received November 21, 2008; revised July 17, 2009; accepted July 24, 2009.