Published OnlineFirst January 16, 2013; DOI: 10.1158/1078-0432.CCR-12-1913

Predictive Biomarkers and Personalized Medicine

Clinical Cancer Research

Massively Parallel Tumor Multigene Sequencing to Evaluate Response to Panitumumab in a Randomized Phase III Study of Metastatic Colorectal Cancer

Marc Peeters[1], Kelly S. Oliner[5], Alex Parker[7], Salvatore Siena[8], Eric Van Cutsem[2], Jing Huang[6], Yves Humblet[3], Jean-Luc Van Laethem[4], Thierry André[9], Jeffrey Wiezorek[5], David Reese[5], and Scott D. Patterson[5]

Abstract

Purpose: To investigate whether EGF receptor (EGFR) pathway mutations predicted response to monotherapy with panitumumab, an anti-EGFR monoclonal antibody, in a randomized phase III study of metastatic colorectal cancer.

Experimental Design: Using massively parallel multigene sequencing, we analyzed 320 samples for 9 genes, with multigene sequence data from 288 (90%) samples.

Results: Mutation rates were: *KRAS* (45%), *NRAS* (5%), *BRAF* (7%), *PIK3CA* (9%), *PTEN* (6%), *TP53* (60%), *EGFR* (1%), *AKT1* (<1%), and *CTNNB1* (2%). In the randomized study and open-label extension, 22 of 138 (16%) wild-type *KRAS* (codons 12/13/61) patients versus 0 of 103 mutant *KRAS* (codons 12/13) patients had objective responses. Of 6 mutant *KRAS* (codon 61) patients, 1 with a Q61H mutation achieved partial response during the extension. Among wild-type *KRAS* (codons 12/13/61) patients, 0 of 9 patients with *NRAS* mutations, 0 of 13 with *BRAF* mutations, 2 of 10 with *PIK3CA* mutations, 1 of 9 with *PTEN* mutations, and 1 of 2 with *CTNNB1* mutations responded to panitumumab. No patients responded to best supportive care alone. Panitumumab treatment was associated with longer progression-free survival (PFS) among wild-type *KRAS* (codons 12/13/61) patients [HR, 0.39; 95% confidence interval (CI), 0.28–0.56]. Among wild-type *KRAS* patients, a treatment effect for PFS favoring panitumumab occurred in patients with wild-type *NRAS* (HR, 0.39; 95% CI, 0.27–0.56) and wild-type *BRAF* (HR, 0.37; 95% CI, 0.24–0.55) but not mutant *NRAS* (HR, 1.94; 95% CI, 0.44–8.44).

Conclusions: These results show the feasibility and potential clinical use of next-generation sequencing for evaluating predictive biomarkers. *Clin Cancer Res; 19(7); 1902–12. ©2012 AACR.*

Introduction

Improvements in progression-free survival (PFS) or objective response rate (ORR) following treatment with the anti–EGF receptor (EGFR) antibodies panitumumab and cetuximab are confined to patients with wild-type *KRAS* tumors as determined by allele-specific real-time PCR (1–6). Current treatment guidelines recommend anti-EGFR monoclonal antibodies for use only in patients with wild-type *KRAS* (codons 12 and 13) colorectal tumors, which are associated with lack of response to these agents (7). However, not all patients with wild-type *KRAS* metastatic colorectal cancer (mCRC) benefit from anti-EGFR antibodies. In addition to *KRAS* mutations, multiple other genes are known to be somatically mutated in mCRC and have been assessed as biomarkers of response to anti-EGFR therapy (8–13). Recent studies have suggested that mutations in *BRAF* (8, 9, 13), as well as mutations in *PI3KCA* and loss of PTEN expression (8, 9, 11–13), may be associated with poor outcome in patients with mCRC treated with agents targeting the EGFR. In a retrospective analysis of tumor samples from patients with mCRC treated with cetuximab plus chemotherapy, mutations in *BRAF*, *NRAS*, and *PIK3CA* exon 20 were associated with low response rates in patients with wild-type *KRAS* tumors (14). Conversely, mutations in the *TP53* tumor suppressor gene have been associated with favorable disease control and time to

Authors' Affiliations: [1]Department of Oncology, Antwerp University Hospital, Edegem; [2]University Hospital Gasthuisberg, Leuven; [3]Centre du Cancer de l'Université Catholique de Louvain; [4]Erasme University Hospital, Brussels, Belgium; [5]Amgen Inc., Thousand Oaks; [6]Amgen Inc., South San Francisco, California; [7]Amgen Inc., Cambridge, Massachusetts; [8]Ospedale Niguarda Cà Granda, Milan, Italy; and [9]Hôpital Saint Antoine and Université Pierre et Marie Curie, Paris, France

Note: Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

M. Peeters, K.S. Oliner, and A. Parker contributed equally to this work.

Current address for A. Parker: Foundation Medicine Inc., Cambridge, MA.

Prior Presentation: These results were presented in part at the 2011 American Society of Clinical Oncology Annual Meeting, June 3–7, Chicago, IL.

Corresponding Author: Marc Peeters, Department of Oncology, Antwerp University Hospital, Wilrijkstraat 10, B-2650 Edegem, Belgium. Phone: 32-3-821-39-54; Fax: 32-3-825-05-64; E-mail: Marc.Peeters@uza.be

doi: 10.1158/1078-0432.CCR-12-1913

Published OnlineFirst January 16, 2013; DOI: 10.1158/1078-0432.CCR-12-1913

Translational Relevance

The anti–EGF receptor (EGFR) monoclonal antibodies panitumumab and cetuximab are recommended for the treatment of wild-type *KRAS* metastatic colorectal cancer (mCRC), but not all patients with wild-type *KRAS* mCRC benefit from their treatment. Other genes have been suggested as potential predictors of outcome in patients treated with anti-EGFR monoclonal antibodies for mCRC. Massively parallel multigene sequencing was used to analyze mutations in *KRAS*, *NRAS*, *EGFR*, *BRAF*, *PTEN*, *PIK3CA*, *AKT*, *TP53*, and *CTNNB1* in tumor samples from patients who received panitumumab in a randomized phase III study or its open-label extension study. Among wild-type *KRAS* patients, a treatment effect for progression-free survival favoring panitumumab occurred in patients with wild-type *NRAS* and wild-type *BRAF* but not mutant *NRAS*. The results of this study, which was intended to generate hypotheses for future prospective studies or meta-analyses, show the feasibility and potential clinical use of next-generation sequencing for evaluating predictive biomarkers.

progression among patients treated with cetuximab plus chemotherapy, including patients with wild-type *KRAS* tumors (10). On the basis of these observations, the strongest hypothesis was that somatic mutations in the *RAS* genes (*KRAS* and *NRAS*) beyond the *KRAS* codons 12 and 13 would be negatively predictive of response to panitumumab. In addition, based on the existing literature, we predicted that activating mutations in *BRAF* and *PIK3CA* and inactivating mutations in *PTEN* would be unfavorable in the context of anti-EGFR therapy, whereas *TP53* mutations would result in a favorable outcome.

We used massively parallel multigene sequencing techniques (also known as next-generation sequencing) to analyze 3 types of alterations in parallel: additional RAS-activating mutations (*KRAS* codon 61; *NRAS* codons 12/13/61), other EGFR signaling pathway genes known to be mutated in mCRC (*EGFR*, *BRAF*, *PTEN*, *PIK3CA*, and *AKT*), and genes known to play a role in colorectal cancer (CRC) tumorigenesis and progression (*TP53* and *CTNNB1*). Massively parallel sequencing technology enables rapid and sensitive sequencing, thus allowing the simultaneous identification of mutations from multiple patient samples in multiple genes potentially important for tumor development (15, 16). The assessment was conducted on banked tissue samples [formalin-fixed paraffin-embedded (FFPE)] that were previously assessed for *KRAS* codons 12 and 13 from patients with mCRC enrolled in a randomized phase III study (the 408 study; ref. 17) and an open-label extension study (the 194 study; ref. 18).

Patients and Methods

Patients

Samples were from patients with metastatic colorectal adenocarcinoma enrolled in the randomized multicenter phase III 408 study (Clinicaltrials.gov, NCT00113763; ref. 17). Patients in the best supportive care (BSC) arm of the randomized phase III study who had radiographically documented disease progression could enroll in the open-label extension 194 study (Clinicaltrials.gov, NCT00113776; ref. 18). The study protocol was approved by independent ethics committees at each participating center. Patients provided written informed consent.

Study design and treatment

Patients enrolled in the randomized phase III study (17) from January 2004 to June 2005 were randomly assigned 1:1 to receive 2-week cycles of intravenous panitumumab 6 mg/kg every 2 weeks plus BSC or BSC alone. Median follow-up time was 35 weeks. Randomization was stratified by Eastern Cooperative Oncology Group (ECOG) performance status (0/1 vs. 2) and region [Western Europe vs. Central/Eastern Europe vs. rest of the world (Canada, Australia, and New Zealand)]. The primary endpoint was PFS. Secondary endpoints included ORR per modified Response Evaluation Criteria in Solid Tumors (RECIST) version 1.0 (19) and overall survival (OS). Local and central reviews were conducted for all assessments.

Patients from the BSC arm of the randomized phase III study who had disease progression could enroll in the crossover extension study (18) and receive intravenous panitumumab 6 mg/kg every 2 weeks plus BSC. In February 2006, median follow-up time was 61 weeks. The primary endpoint was safety. Exploratory endpoints included PFS, ORR per modified RECIST version 1.0 (19), time to response, duration of response, duration of stable disease, and OS, all per local review.

Biomarker analysis

Archival CRC samples (all obtained from the patients before trial initiation) were previously analyzed for *KRAS* mutations (codons 12 and 13) using allele-specific PCR (DxS/Qiagen; ref. 1). For this analysis, tumor DNA was extracted from additional archival FFPE tissue sections using the QIAmp DNA FFPE Tissue Kit (Qiagen). One specimen per patient was analyzed. DNA isolates were quantified using the Quant-iT PicoGreen dsDNA reagent (Invitrogen). Slides representing the primary tumors of 324 patients were available for analysis. Two slides were available in most cases ($n = 308$), but only a single slide was available for 11 patients, and 3 or more slides were available for 5 patients. Sequencing libraries were generated and used in 454 GS FLX (Roche 454 Life Sciences) sequencing experiments. The PCR primers in this analysis targeted exons 2 and 3 of *NRAS*; exon 3 of *KRAS*; exon 2 of *CTNNB1*; exon 15 of *BRAF*; exon 3 of *AKT1*; exons 1, 2, 9, 10, and 20 of *PIK3CA*; exons 17 through 22 of *EGFR*; and all exons of *PTEN* and *TP53* (primer pair sequences are presented in Supplementary Table S1).

Up to 24 patient samples were interrogated per sequencing experiment. Amplified sequencing libraries were evaluated by capillary electrophoresis using the Agilent 2100 Bioanalyzer and DNA1000 Kit (Agilent Technologies, Inc.)

Published OnlineFirst January 16, 2013; DOI: 10.1158/1078-0432.CCR-12-1913

before sequencing. Libraries showing little or no amplification of target amplicons, and/or a molar excess of nonspecific PCR artifact relative to target amplicons (again using the Agilent 2100 Bioanalyzer and DNA1000 Kit), were not used for sequencing. Sequence data were basecalled and filtered using default 454 GS FLX software settings and further filtered to reject sequences that did not contain 2 complete cognate locus-specific primer sequences (i.e., the forward and reverse primer sequences for 1 of the intended PCR amplicons). The latter filter prevented sequences arising from PCR artifacts being included in data used for mutation analysis.

Mutation analysis was carried out using the 454 Amplicon Variant Analysis (AVA) software version 2.0 (Roche 454 Life Sciences). Gene–subject combinations in which a mutation was observed were assigned mutant status, and wild-type status was assigned to gene–subject combinations in which (i) no mutations were observed, and (ii) the median number of sequence reads per amplicon for that gene in that subject was more than 40. If no mutation was observed but this second criterion was not met, the status of that gene–subject intersection was classified as "not determined." Some FFPE DNA samples, when amplified by PCR, exhibit a sharply elevated frequency of artifactual transition substitutions. To avoid making false-positive mutation identifications in these cases, a metric of the severity of this phenomenon was derived by counting the number of nonreference bases present at each of 2,889 nucleotide positions within the panel in which a substitution would not alter the encoded polypeptide or disrupt a splice donor or acceptor sequence and for which there was no reported single-nucleotide polymorphism. Subsequently, a sample-specific cutoff for acceptance of putative missense or nonsense sequence variants was applied such that any variant present in a smaller fraction of sequence reads than the third most frequent "silent position" in a given sample was not included (i.e., variants with approximately >0.1% probability of being an artifact). The sample-specific cutoff was generally less than 5%.

The analysts conducting the assays were blinded to clinical outcome data. The multigene sequencing assays were extensively qualified to show the ability to confidently detect mutations at a level of 5% of sequence reads per amplicon using both cell line DNA and procured FFPE colorectal tumor samples. Because of the nature of this qualification, the demonstration of correlation between this sequencing methodology and bidirectional sequencing (20), the lack of commercially available validated assays for all of these mutations and the limiting amount of material available for these samples, confirmation of the mutation status using an additional method was not pursued.

Statistical analysis

The analysis included all intent-to-treat patients with available biomarker information. The statistical analysis plan was developed before analyses were conducted. As specified in the analysis plan, patient data were analyzed on the basis of randomized treatment assignment (regardless of actual treatment administered).

Assessment of gene frequency

Genotype frequency was calculated for each biomarker by treatment arm and by overall frequency.

Evaluation of treatment effect on PFS in the randomized study

In the randomized phase III study, the treatment effect on PFS (independent blinded radiologic review) of panitumumab plus BSC versus BSC for all evaluable patients and the wild-type *KRAS* subgroup was estimated by genotype using a Cox proportional hazards model adjusted for the randomization factors.

Evaluation of biomarker effect on PFS in the randomized study

The relative biomarker effect (mutant vs. wild-type) within each treatment arm (panitumumab plus BSC, BSC only, and total) was evaluated using a Cox proportional hazards model adjusted for the randomization factors.

Analysis of the randomized study and the extension studies combined

This analysis included patients enrolled in the panitumumab plus BSC arm of the randomized phase III study and in the open-label extension study. The analysis was conducted for each study and for both studies combined, for all evaluable patients, and within the wild-type *KRAS* subgroup. For the combined analyses, the patients enrolled in the extension study were considered equivalent to patients randomized to panitumumab in the phase III study. Because the extension study was reviewed locally, ORR and PFS based on local review were used for analyses of the randomized study and the extension study.

PFS was defined as time from the date of enrollment to disease progression or death. Patients who had not progressed or died during the study were censored at the last evaluable disease assessment date. For the panitumumab plus BSC arm in the randomized phase III study, the initial enrollment date was used. For patients in the extension study, enrollment date into the extension study was used. Because the key eligibility criteria for the extension study was disease progression in the randomized study, by definition, patients in the extension study already had one disease progression before entering the extension study.

ORR and 95% confidence intervals (CI) were calculated for each biomarker by genotype and by study: the panitumumab plus BSC arm in the randomized study, the extension study, and both combined. Cox proportional hazard models adjusted for the randomization factors ECOG score and demographic region were used to estimate the effect of mutant versus wild-type of each biomarker on PFS under the panitumumab plus BSC treatment. For these exploratory analyses, the reported *P* values are presented for descriptive purposes only. *P* values near or less than 0.05 are believed to show potential for further investigation (i.e.,

Published OnlineFirst January 16, 2013; DOI: 10.1158/1078-0432.CCR-12-1913

for hypothesis generation). No adjustments for multiple comparisons were made with the reported *P* values.

Results

Patients

As previously reported, 463 patients were randomized to panitumumab 6 mg/kg every 2 weeks plus BSC ($n = 231$) or BSC alone ($n = 232$) in the phase III study (study 20020408); 176 patients (76%) from the BSC arm with progressive disease enrolled in the optional extension study (study 20030194) of panitumumab monotherapy (Fig. 1; refs. 17, 18). In the randomized study, PFS was improved with panitumumab compared with BSC alone (HR, 0.54; 95% CI, 0.44–0.66; $P < 0.0001$; ref. 17). The treatment effect of panitumumab was greater ($P < 0.0001$) among patients with wild-type *KRAS* codons 12 and 13 (HR, 0.45; 95% CI, 0.34–0.59) than among those with *KRAS* mutations (HR, 0.99; 95% CI, 0.73–1.36; ref. 1). The ORR among patients with wild-type *KRAS* (codons 12/13) who were randomized to panitumumab was 17% (95% CI, 11%–25%); no responses occurred among patients with *KRAS* mutations (1). No significant difference in OS was observed between the treatment arms in the randomized study (HR, 1.00; 95% CI, 0.82–1.22; $P = 0.81$) and *KRAS* status was not predictive for OS (1, 17), perhaps because 76% of patients from the BSC arm received panitumumab in the extension study.

Gene mutation analysis

Overall, 320 archival FFPE tumor samples were available from the 463 patients in the 20020408 study, 288 (288 of 320; 90%) of which (panitumumab, $n = 147$; BSC, $n = 141$; Fig. 1) provided information for multiple genes. Demographic and clinical characteristics for patients with or without multigene information were generally similar; patients from Western Europe were more likely to have available multigene information (Supplementary Table S2). Among the 141 patients randomized to the BSC arm with samples, 110 were enrolled in the panitumumab extension study. Forty-nine primer pairs were used to amplify 41 exons, resulting in 5.7×10^6 DNA sequence reads, comprising 1.26×10^9 base pairs. Estimated total tissue volume [area × (number of slides) × 0.005] used for DNA isolation ranged from less than 0.1 to more than 9 mm^3 (mean, 3.1 mm^3), and the quantity of DNA recovered ranged from 0.1 to 5.37 μg (mean, 1.19 μg). Consistent with studies that have shown amplification of *KRAS* sequences from small quantities of FFPE tissue (21), there was only a weak relationship between amount of tissue processed and DNA yield ($R^2 = 0.16$).

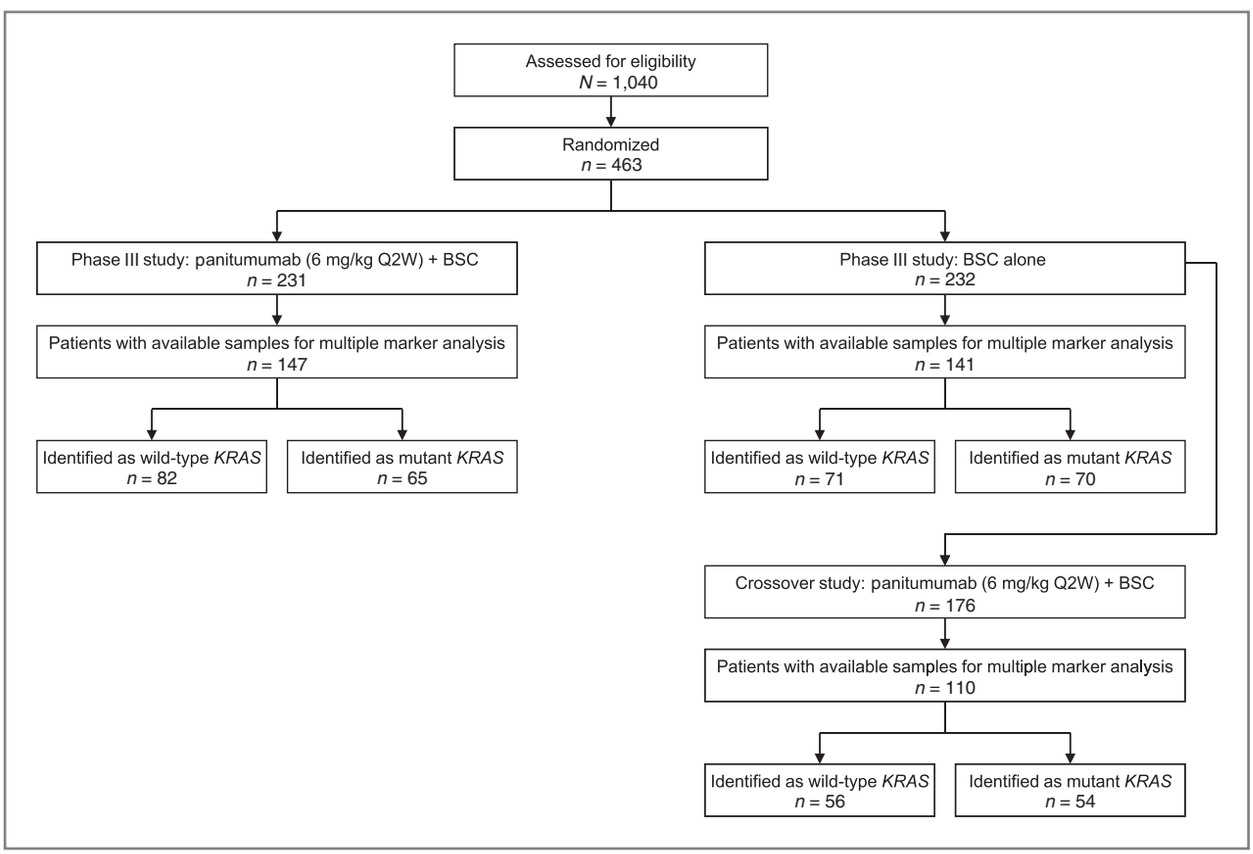

Figure 1. CONSORT diagram. Q2W, every 2 weeks.

Published OnlineFirst January 16, 2013; DOI: 10.1158/1078-0432.CCR-12-1913

Table 1. Mutation rates in patient tumor specimens available for analysis ($N = 288$)

Gene	Mutation rate, %	Patients with mutations, *n*	Patients with data available, *n*	Data completeness[a], %
KRAS (codons 12, 13, and 61)	45	124	277	96
KRAS (codons 12 and 13)[b]	42	117	280	97
KRAS (codon 61)	2	7	284	99
NRAS	5	14	282	98
EGFR	1	3	280	97
BRAF	7	18	243	84
PTEN	6	15	272	94
PIK3CA	9	24	255	89
AKT1	<1	1	250	87
TP53	60	167	277	96
CTNNB1	2	5	256	89

NOTE: *AKT1*, v-akt murine thymoma viral oncogene homolog 1; *BRAF*, v-raf murine sarcoma viral oncogene homolog B1; *CTNNB1*, catenin (cadherin-associated protein), β-1, 88 kDa; *KRAS*, v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog; *NRAS*, neuroblastoma RAS viral oncogene homolog; *PIK3CA*, phosphoinositide-3-kinase, catalytic, α-polypeptide; *PTEN*, phosphatase and tensin homolog; *TP53*, tumor protein p53.

[a]For each gene, data completeness assessed as the number of patients with data available divided by 288 (the number of patients with samples yielding results).

[b]Data from a previous analysis of *KRAS* mutations (codons 12 and 13) using allele-specific polymerase chain reaction (DxS/Qiagen; ref. 1).

Mutation rates and data completeness for each of the genes analyzed (*KRAS*, *NRAS*, *EGFR*, *BRAF*, *PTEN*, *PIK3CA*, *AKT1*, *TP53*, and *CTNNB1*) are shown in Table 1 and mutations identified for individual patients are shown in Supplementary Table S3. Overall, 124 of 277 (45%) patients with available data had a mutation in *KRAS*, among whom 117 had mutations in *KRAS* codons 12 or 13, and 7 had mutations in *KRAS* codon 61. Five percent of patients had mutations in *NRAS* and 7% had mutations in *BRAF*. Sequencing identified 109 tumors with mutations in more than 1 gene and 20 tumors with more than 1 mutation in a single gene. Three tumors were identified with mutations in *KRAS* (codons 12/13/61) and *NRAS*, and 2 tumors were identified with mutations in both *KRAS* (codons 12/13/61) and *BRAF* (Table 2). Most patients with 2 or more mutant genes had a *TP53* mutation and a mutation at another locus (or 2 other loci). Dual mutations in genes other than *TP53* were observed in several patients.

Table 2. Joint frequency distribution of mutations across all gene pairs in patient tumor specimens available for analysis ($N = 288$)

Gene, n	*AKT1*, $n = 1$	*BRAF*, $n = 18$	*CTNNB1*, $n = 5$	*EGFR*, $n = 3$	*KRAS*, $n = 124$	*NRAS*, $n = 14$	*PIK3CA*, $n = 24$	*PTEN*, $n = 15$	*TP53*, $n = 167$
AKT1									
BRAF	1								
CTNNB1	—	—							
EGFR	—	—	—						
KRAS[a]	—	2	1	3					
NRAS	—	–	—	—	3				
PIK3CA	—	2	1	—	13	—			
PTEN	—	4	—	1	6	1	1		
TP53	—	8	1	1	66	9	12	9	

NOTE: *AKT1*, v-akt murine thymoma viral oncogene homolog 1; *BRAF*, v-raf murine sarcoma viral oncogene homolog B1; *CTNNB1*, catenin (cadherin-associated protein), β-1, 88 kDa; *KRAS*, v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog; *NRAS*, neuroblastoma RAS viral oncogene homolog; *PIK3CA*, phosphoinositide-3-kinase, catalytic, α-polypeptide; *PTEN*, phosphatase and tensin homolog; *TP53*, tumor protein p53.

[a]*KRAS* mutations in codons 12, 13, or 61.

Published OnlineFirst January 16, 2013; DOI: 10.1158/1078-0432.CCR-12-1913

Association between gene mutations and PFS among patients with wild-type *KRAS* in the randomized study

The effect of panitumumab treatment on PFS (independent blinded radiologic review) for each genotype in patients with wild-type *KRAS* from the randomized study is shown in Fig. 2. Among patients with wild-type *KRAS* (codons 12/13/61) and wild-type *NRAS* ($n = 138$), treatment with panitumumab was associated with improved PFS (HR, 0.39; 95% CI, 0.27–0.56; $P < 0.001$), but in patients with wild-type *KRAS* (codons 12/13/61) and mutant *NRAS* ($n = 11$), treatment with panitumumab was not associated with longer PFS (HR, 1.94; 95% CI, 0.44–8.44; $P = 0.379$). Among wild-type *KRAS* patients, a Cox proportional hazards model with *NRAS*, treatment, and their interaction showed $P = 0.076$ for the interaction term, which was suggestive of an interaction. However, the presence of *BRAF* mutations among patients with wild-type *KRAS* did not seem to predict the effect of treatment with panitumumab on PFS. A favorable effect of panitumumab on PFS was observed in patients with wild-type *BRAF* ($n = 115$; HR, 0.37; 95% CI, 0.24–0.55; $P < 0.001$) and potentially in patients with *BRAF* mutations ($n = 15$; HR, 0.34; 95% CI, 0.09–1.24; $P = 0.104$). Similarly, a favorable effect of panitumumab on PFS was

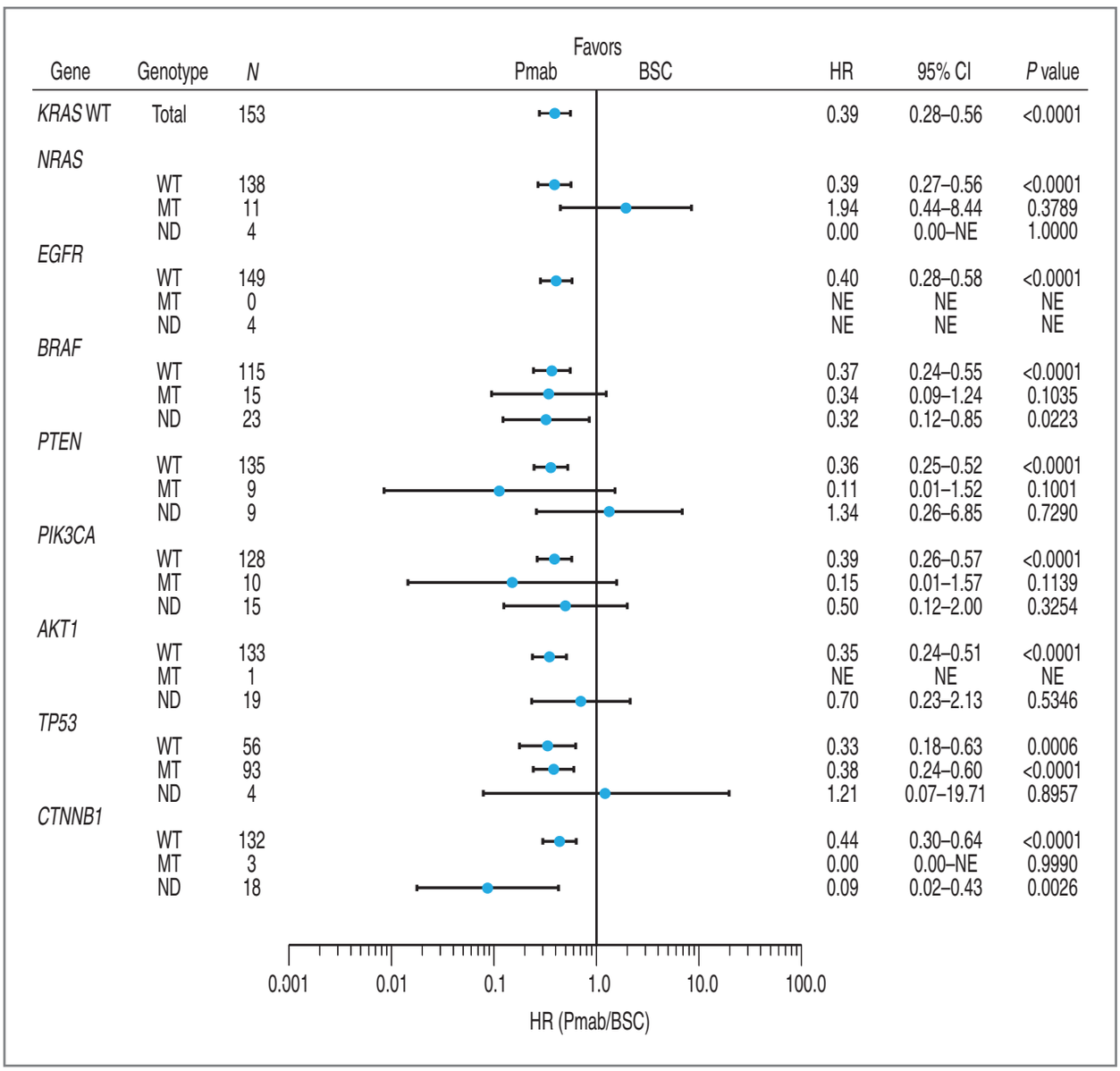

Figure 2. HRs for the relative risk of PFS in the *KRAS* wild-type subgroup for panitumumab plus BSC versus BSC alone. MT, mutant; ND, not determined; NE, not evaluable; Pmab, panitumumab; WT, wild-type.

Published OnlineFirst January 16, 2013; DOI: 10.1158/1078-0432.CCR-12-1913

observed in patients with wild-type *PTEN* ($n = 135$; HR, 0.36; 95% CI, 0.25–0.52; $P < 0.001$) and in patients with wild-type *PIK3CA* ($n = 128$; HR, 0.39; 95% CI, 0.26–0.57; $P < 0.001$). Trends toward improved PFS among patients randomized to panitumumab were observed for patients with *PTEN* mutations ($n = 9$; HR, 0.11; 95% CI, 0.01–1.52; $P = 0.100$) and in patients with *PIK3CA* mutations ($n = 10$; HR, 0.15; 95% CI, 0.01–1.57; $P = 0.114$). Cox proportional hazard models similar to those described earlier for *NRAS* were conducted for *BRAF*, *PTEN*, and *PIK3CA*. In each case, the interaction terms did not suggest statistical significance (*BRAF*, $P = 0.850$; *PTEN*, $P = 0.771$; *PIK3CA*, $P = 0.616$).

Among patients with wild-type *KRAS* from the randomized study, the presence of *BRAF* mutations was associated with poor PFS regardless of treatment. Among patients with *BRAF* mutations, the HR for PFS for mutant versus wild-type status was 2.55 (95% CI, 1.04–6.24) in the BSC arm, 3.27 (95% CI, 1.52–7.01) in the panitumumab plus BSC arm, and 2.39 (95% CI, 1.36–4.21) overall (Fig. 3). No other gene mutation among patients with wild-type *KRAS* was negatively prognostic for PFS.

Association between gene mutations and objective response among panitumumab-treated patients with wild-type *KRAS* in the randomized and extension studies

The sample size was increased by examining the ORR in patients treated with panitumumab monotherapy in both the randomized and the extension studies. ORR was 16% for patients with wild-type *KRAS* ($n = 138$) versus 1% with mutant-type *KRAS* (codons 12/13/61; $n = 109$). One patient with a *KRAS* Q61H mutation had a partial response (local review) while receiving panitumumab in the extension study. Fifty-eight percent of this patient's *KRAS* codon 61 sequencing reads were Q61H. Furthermore, a mutation in *TP53* was detected in this patient's sample; all other genes examined were wild-type.

ORRs for wild-type versus mutant genotypes among patients with wild-type *KRAS* (codons 12/13/61) who were randomized to panitumumab in the randomized study or the extension study are summarized in Table 3 and the Supplementary Figure. Among 126 patients with wild-type *KRAS* (codons 12/13/61) and *NRAS*, 22 achieved objective responses with an ORR of 17% (95% CI, 0.11–0.25). Among 98 patients with wild-type *KRAS*, *NRAS*, and *BRAF*, 18 achieved objective responses with an ORR of 18% (95% CI, 0.11–0.27). No responses to panitumumab occurred in patients with wild-type *KRAS* and mutations in *NRAS* or *BRAF* (0 of 22 patients; 95% CI, 0–0.15). No patients responded to BSC alone.

Discussion

To our knowledge, this is the first use of next-generation sequencing to assess potential predictive biomarkers of response using tumor samples from a randomized phase III clinical trial. Previous reports described the use of massively parallel pyrosequencing techniques to investigate disease markers only in small patient populations (22–24).

In this retrospective analysis designed to explore effects of tumor genotype on outcomes, we were able to show trends and develop hypotheses; however, confidence of the observed trends was somewhat limited by the low prevalence of many of the gene mutations. For example, *BRAF* is mutated in approximately 10% of patients with mCRC. In this study, the treatment HR or effect size was calculated to be 0.34 (95% CI, 0.09–1.24), similar to that of wild-type *KRAS*. Using this HR, 46 specimens that are *KRAS* wild-type and *BRAF* mutant would be required to achieve 90% power with 2-sided type I error set to 0.05. For the *KRAS* wild-type/*NRAS*-mutant population, given an HR of 1.94 (95% CI, 0.44–8.44), we estimate that 129 patients with this genotype would be required to provide 90% power. Given these

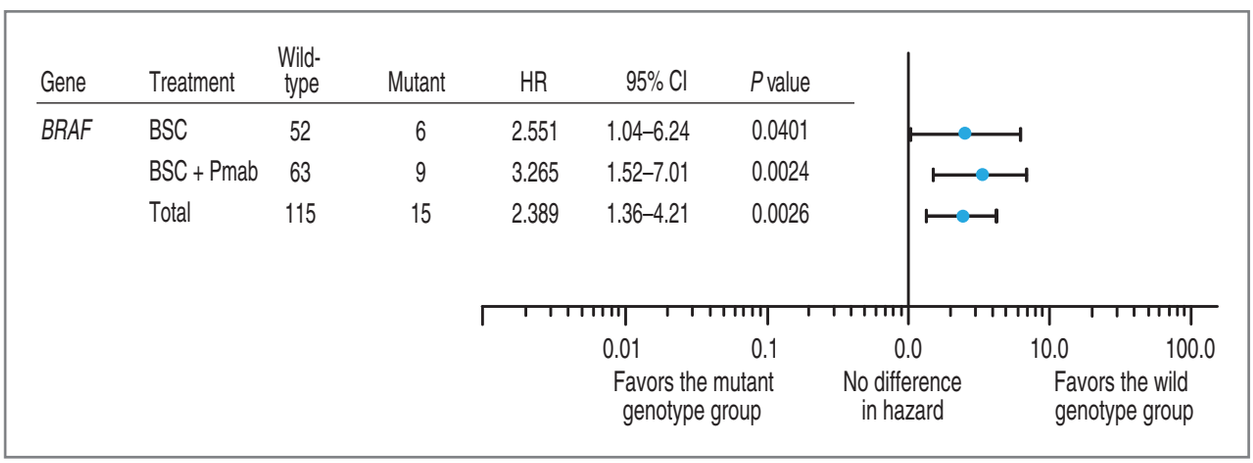

Figure 3. HRs for the relative risk of PFS with panitumumab therapy in the *KRAS* wild-type subgroup for wild-type versus mutant genotype in the randomized phase III and extension studies combined. Pmab, panitumumab.

Published OnlineFirst January 16, 2013; DOI: 10.1158/1078-0432.CCR-12-1913

Table 3. Response rates of patients with wild-type *KRAS* (codons 12/13/61) who were randomized to panitumumab plus BSC[a]

		Randomized phase III study panitumumab + BSC $n = 82$		Extension study panitumumab + BSC $n = 56$		Combined panitumumab + BSC $n = 138$	
Genotype		*n*	Response rate, % (95% CI)	*n*	Response rate, % (95% CI)	*n*	Response rate, % (95% CI)
NRAS	WT	76	13 (6–23)	50	24 (13–38)	126	17 (11–25)
	MT	4	0 (0–60)	5	0 (0–52)	9	0 (0–34)
EGFR	WT	82	12 (6–21)	52	23 (13–37)	134	16 (11–24)
	MT	0	NA	0	NA	0	NA
BRAF	WT	63	14 (7–25)	44	21 (10–35)	107	17 (10–25)
	MT	9	0 (0–34)	4	0 (0–60)	13	0 (0–25)
PTEN	WT	72	13 (6–22)	50	22 (12–36)	122	16 (10–24)
	MT	7	14 (0–58)	2	0 (0–84)	9	11 (0–48)
PIK3CA	WT	74	12 (6–22)	43	19 (8–33)	117	15 (9–22)
	MT	5	20 (1–72)	5	20 (1–72)	10	20 (3–56)
AKT1	WT	69	15 (7–25)	52	19 (10–33)	121	17 (10–24)
	MT	1	0 (0–98)	0	NA	1	0 (0–98)
TP53	WT	32	16 (5–33)	18	11 (1–35)	50	14 (6–27)
	MT	49	10 (3–22)	35	26 (13–43)	84	17 (9–26)
CTNNB1	WT	72	11 (5–21)	46	22 (11–36)	118	15 (9–23)
	MT	2	50 (1–99)	0	NA	2	50 (1–99)

NOTE: *AKT1*, v-akt murine thymoma viral oncogene homolog 1; *BRAF*, v-raf murine sarcoma viral oncogene homolog B1; *CTNNB1*, catenin (cadherin-associated protein), β-1, 88 kDa; MT, mutant; NA, not available; *NRAS*, neuroblastoma RAS viral oncogene homolog; *PIK3CA*, phosphoinositide-3-kinase, catalytic, α-polypeptide; *PTEN*, phosphatase and tensin homolog; *TP53*, tumor protein p53; WT, wild-type.

[a]Per local review.

limitations, hypothesis testing of these low prevalence genes will likely require carefully constructed meta-analyses. The use of multiplexed, multigene analysis is a useful tool for hypothesis generation in translational studies.

The frequency of gene mutations observed in this study with 5% sensitivity is generally consistent with mutation rates for adenocarcinoma of the colon or rectum published in the Catalogue of Somatic Mutations in Cancer database (25) and with those reported by Vaughn and colleagues (26). The consistency of our results with previously reported studies suggests that the data are reliable.

Consistent with other reports of anti-EGFR antibodies in mCRC (1–3, 6, 14, 27), objective responses to panitumumab were confined to patients with wild-type *KRAS* tumors. Interestingly, 1 patient with a codon 61 mutation (Q61H) had a partial response per local review. Prior analyses of *KRAS* mutational status in anti-EGFR monoclonal antibody therapy have primarily assessed mutations only in codons 12 and 13 (1, 3, 6, 14, 27); however, 1 study reported that no patients with mutations in codon 61 ($n = 8$) responded to treatment with cetuximab plus irinotecan (28). We did not assess the predictive or prognostic value of individual codon 12/13 mutations but a recent pooled analysis of 3 randomized phase III studies of panitumumab in mCRC (which included patients from the randomized phase III 408 study), found that no individual mutant *KRAS* allele was consistently associated with PFS or OS outcomes (29). Conversely, pooled analyses of clinical trials in which cetuximab was administered as a component of first-line chemotherapy (30) or in chemotherapy-refractory disease (31) have suggested that patients with colorectal tumors bearing *KRAS* G13D mutations have favorable outcomes versus patients with other *KRAS* mutations.

Our data are consistent with the hypothesis that *NRAS* mutations, which occur infrequently (<5%) in mCRC (14, 25, 26, 32, 33), may limit the efficacy of panitumumab. In patients with wild-type *KRAS* and mutant *NRAS* tumors assigned to panitumumab therapy in either the randomized or extension study ($n = 9$), there was a lack of response (Table 3) and a lack of improved PFS (HR for mutant vs. wild-type, 1.71; 95% CI, 0.81–3.62). As described earlier, because mutations in *NRAS* are low-prevalence mutations, their true predictive or prognostic value must be confirmed in larger studies.

The negative prognostic effect of *BRAF* is clearly seen for PFS (Fig. 3). Because no patients responded in the BSC arm of the randomized study, evaluation of its prognostic potential for ORR is difficult. However, the negative effect of *BRAF* mutation was clearly seen in the treatment arm within the

Published OnlineFirst January 16, 2013; DOI: 10.1158/1078-0432.CCR-12-1913

KRAS wild-type population, in which the response rate was 17% with wild-type *BRAF* compared with no response with mutant *BRAF*. In addition, there is ample evidence that *BRAF* mutations may have use as a prognostic marker in mCRC (14, 34–38). In this analysis, it is unknown whether the observed dual mutations in *KRAS* and *BRAF* arose from tumor heterogeneity (i.e., different cell populations within the tumor) or the presence of both mutations within individual tumor cells. The use of techniques capable of discrimination at the single-cell level will be required to address this question.

Our results do not support the hypothesis that *PIK3CA* or *PTEN* mutations are negative predictive markers as has been suggested by the work of Sartore-Bianchi and colleagues (39). In the randomized and extension studies, 18 patients with wild-type *KRAS* (codons 12/13/61) treated with panitumumab monotherapy had *PIK3CA* and/or *PTEN* mutations, and of these, 3 patients (*PTEN* mutation, $n = 1$; *PIK3CA* mutation, $n = 2$) achieved a partial response. In addition, 1 of these 3 patients had an exon 20 *PIK3CA* mutation. Our observations are not consistent with the hypothesis that exon 20 *PIK3CA* mutations (but not exon 9 *PIK3CA* mutations) predict response to treatment with anti-EGFR monoclonal antibodies (14). De Roock and colleagues previously reported that although *PIK3CA* exon 9 mutations did not influence outcomes among patients receiving cetuximab-based chemotherapy for mCRC, exon 20 mutations were associated with a lower response rate (0% vs. 37% for wild-type *PIK3CA*; $P = 0.029$) and disease control rate (33% vs. 76%; $P = 0.0078$), as well as shorter median PFS (11.5 vs. 24 weeks; $P = 0.013$) and OS (34 vs. 51 weeks; $P = 0.0057$). However, it is possible that our results may have been affected by the smaller number of patients with *PIK3CA* data evaluable for response in the phase III study ($n = 79$) and extension study ($n = 48$) compared with the De Roock and colleagues analysis ($n = 339$). Although, we found favorable effects of panitumumab on PFS in patients with wild-type and mutant *PTEN* and *PIK3CA*, our results should be interpreted with caution given the small number of patients with these mutations. This exploratory analysis suggests that other mutated genes within the EGFR signaling pathway do not seem to confer the same strictly negative predictive value for response to anti-EGFR antibody therapy that specific mutations in the RAS family genes (*KRAS* and *NRAS*) seem to confer.

Future meta-analyses would ideally use the same well-established methodology to ensure confidence and consistency in the wild-type and mutation assignments. This is especially important due to the varying tumor content of each tissue section, as well as the potential for heterogeneity within tumor cells of the individual tissue section. It should be noted that when more than 1 mutation is present in a tissue section extract, the percentage of mutant sequence reads is not always the same across the different amplimers, thus reflecting either aneuploidy of the different gene segments or tumor cell heterogeneity (data not shown). It should also be noted that we did not confirm mutations using a second analytic technique. However, in a separate comparability study, we found assessment of *KRAS* status using Roche 454 pyrosequencing to have a high level of agreement ($\kappa = 0.94$) with direct sequencing (20).

In summary, although only *KRAS* mutational status predicted response to treatment with panitumumab, among patients with wild-type *KRAS*, objective responses did not occur in patients with mutations in *NRAS* or *BRAF*. Because of the relatively limited frequencies of mutations in *KRAS* codon 61 and *NRAS* codons 12/13/61, a large prospective analysis of these biomarkers will be difficult to conduct. However, because these results show the use of retrospective analysis of tumor specimens using massively parallel multigene sequencing, a pooled analysis of gene mutations in the RAS family is warranted.

Disclosure of Potential Conflicts of Interest

M. Peeters and Y. Humblet have honoraria from Speakers Bureau and are consultant/advisory board members of Amgen, Inc. K.S. Oliner, J. Huang, J. Wiezorek, D. Reese, and S.D. Patterson are employees of Amgen, Inc. and have ownership interest (including patents) in the same. A. Parker was an employee of Amgen, Inc. at the time the study was conducted. T. Andre has honoraria from Speakers Bureau of Amgen, Inc. and Merck Serono and is a consultant/advisory board member of Roche. No potential conflicts of interest were disclosed by the other authors.

Authors' Contributions

Conception and design: M. Peeters, K.S. Oliner, A. Parker, S. Siena, E. Van Cutsem, J. Wiezorek, D. Reese, S.D. Patterson
Development of methodology: K.S. Oliner, A. Parker, E. Van Cutsem, J. Wiezorek, S.D. Patterson
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): M. Peeters, K.S. Oliner, A. Parker, S. Siena, E. Van Cutsem, Y. Humblet, J.-L. Van Laethem, T. André, S.D. Patterson
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): M. Peeters, K.S. Oliner, A. Parker, S. Siena, E. Van Cutsem, J. Huang, Y. Humblet, J.-L. Van Laethem, T. André, J. Wiezorek, D. Reese, S.D. Patterson
Writing, review, and/or revision of the manuscript: M. Peeters, K.S. Oliner, A. Parker, S. Siena, E. Van Cutsem, J. Huang, Y. Humblet, J.-L. Van Laethem, T. André, J. Wiezorek, D. Reese, S.D. Patterson
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): K.S. Oliner
Study supervision: M. Peeters, E. Van Cutsem, D. Reese

Acknowledgments

The authors thank the extended Clinical Study Team that conducted the extensive measures that allowed sample analysis (Simon Collins, Jennifer Hawkins, Mark Ekdahl, Feng Hong, and Carol Johnson) as well as the Amgen Massachusetts Molecular Sciences Laboratory, particularly Sunita Badola, Kim Tsui, and Lauren Young for generating the raw sequencing data. The authors also thank Ying Cheng and Lixia Zhu (i3 Statprobe) for their extensive programming efforts, and Ali Hassan and Benjamin Scott (Complete Healthcare Communications, Inc., Chadds Ford, PA) for assistance in the preparation of this article.

Grant Support

This study was funded by Amgen Inc.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received June 25, 2012; revised December 5, 2012; accepted December 19, 2012; published OnlineFirst January 16, 2013.

Published OnlineFirst January 16, 2013; DOI: 10.1158/1078-0432.CCR-12-1913

References

1. Amado RG, Wolf M, Peeters M, Van Cutsem E, Siena S, Freeman DJ, et al. Wild-type *KRAS* is required for panitumumab efficacy in patients with metastatic colorectal cancer. J Clin Oncol 2008;26:1626–34.
2. Douillard JY, Siena S, Cassidy J, Tabernero J, Burkes R, Barugel M, et al. Randomized, phase III trial of panitumumab with infusional fluorouracil, leucovorin, and oxaliplatin (FOLFOX4) versus FOLFOX4 alone as first-line treatment in patients with previously untreated metastatic colorectal cancer: the PRIME study. J Clin Oncol 2010;28:4697–705.
3. Peeters M, Price TJ, Cervantes A, Sobrero AF, Ducreux M, Hotko Y, et al. Randomized phase III study of panitumumab with fluorouracil, leucovorin, and irinotecan (FOLFIRI) compared with FOLFIRI alone as second-line treatment in patients with metastatic colorectal cancer. J Clin Oncol 2010;28:4706–13.
4. Karapetis CS, Khambata-Ford S, Jonker DJ, O'Callaghan CJ, Tu D, Tebbutt NC, et al. K-ras mutations and benefit from cetuximab in advanced colorectal cancer. N Engl J Med 2008;359:1757–65.
5. Bokemeyer C, Bondarenko I, Makhson A, Hartmann JT, Aparicio J, de Braud F, et al. Fluorouracil, leucovorin, and oxaliplatin with and without cetuximab in the first-line treatment of metastatic colorectal cancer. J Clin Oncol 2009;27:663–71.
6. Van Cutsem E, Köhne CH, Hitre E, Zaluski J, Chang Chien CR, Makhson A, et al. Cetuximab and chemotherapy as initial treatment for metastatic colorectal cancer. N Engl J Med 2009;360:1408–17.
7. NCCN clinical practice guidelines in oncology: colon cancer v.3.2012. Fort Washington, PA: National Comprehensive Cancer Network; 2012.
8. Bardelli A, Siena S. Molecular mechanisms of resistance to cetuximab and panitumumab in colorectal cancer. J Clin Oncol 2010;28:1254–61.
9. De Roock W, Biesmans B, De Schutter J, Tejpar S. Clinical biomarkers in oncology: focus on colorectal cancer. Mol Diagn Ther 2009;13:103–14.
10. Oden-Gangloff A, Di Fiore F, Bibeau F, Lamy A, Bougeard G, Charbonnier F, et al. TP53 mutations predict disease control in metastatic colorectal cancer treated with cetuximab-based chemotherapy. Br J Cancer 2009;100:1330–5.
11. Perkins G, Lievre A, Ramacci C, Meatchi T, de Reynies A, Emile JF, et al. Additional value of EGFR downstream signaling phosphoprotein expression to KRAS status for response to anti-EGFR antibodies in colorectal cancer. Int J Cancer 2010;127:1321–31.
12. Jhawer M, Goel S, Wilson AJ, Montagna C, Ling YH, Byun DS, et al. PIK3CA mutation/PTEN expression status predicts response of colon cancer cells to the epidermal growth factor receptor inhibitor cetuximab. Cancer Res 2008;68:1953–61.
13. Sood A, McClain D, Maitra R, Basu-Mallick A, Seetharam R, Kaubisch A, et al. PTEN gene expression and mutations in the PIK3CA gene as predictors of clinical benefit to anti-epidermal growth factor receptor antibody therapy in patients with KRAS wild-type metastatic colorectal cancer. Clin Colorectal Cancer 2012;11:143–50.
14. De Roock W, Claes B, Bernasconi D, De Schutter J, Biesmans B, Fountzilas G, et al. Effects of KRAS, BRAF, NRAS, and PIK3CA mutations on the efficacy of cetuximab plus chemotherapy in chemotherapy-refractory metastatic colorectal cancer: a retrospective consortium analysis. Lancet Oncol 2010;11:753–62.
15. Brenner BM, Rosenberg D. High-throughput SNP/CGH approaches for the analysis of genomic instability in colorectal cancer. Mutat Res 2010;693:46–52.
16. Morozova O, Marra MA. From cytogenetics to next-generation sequencing technologies: advances in the detection of genome rearrangements in tumors. Biochem Cell Biol 2008;86:81–91.
17. Van Cutsem E, Peeters M, Siena S, Humblet Y, Hendlisz A, Neyns B, 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;25:1658–64.
18. Van Cutsem E, Siena S, Humblet Y, Canon JL, Maurel J, Bajetta E, et al. An open-label, single-arm study assessing safety and efficacy of panitumumab in patients with metastatic colorectal cancer refractory to standard chemotherapy. Ann Oncol 2008;19:92–8.
19. Therasse P, Arbuck SG, Eisenhauer EA, Wanders J, Kaplan RS, Rubinstein L, et al. New guidelines to evaluate the response to treatment in solid tumors. European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. J Natl Cancer Inst 2000;92:205–16.
20. Oliner K, Juan T, Suggs S, Wolf M, Sarosi I, Freeman DJ, et al. A comparability study of 5 commercial KRAS tests. Diagn Pathol 2010;5:23.
21. Kotoula V, Charalambous E, Biesmans B, Malousi A, Vrettou E, Fountzilas G, et al. Targeted KRAS mutation assessment on patient tumor histologic material in real time diagnostics. PLoS ONE 2009;4:e7746.
22. Korshunova Y, Maloney RK, Lakey N, Citek RW, Bacher B, Budiman A, et al. Massively parallel bisulphite pyrosequencing reveals the molecular complexity of breast cancer-associated cytosine-methylation patterns obtained from tissue and serum DNA. Genome Res 2008;18:19–29.
23. Beck J, Urnovitz HB, Mitchell WM, Schutz E. Next generation sequencing of serum circulating nucleic acids from patients with invasive ductal breast cancer reveals differences to healthy and nonmalignant controls. Mol Cancer Res 2010;8:335–42.
24. Timmermann B, Kerick M, Roehr C, Fischer A, Isau M, Boerno ST, et al. Somatic mutation profiles of MSI and MSS colorectal cancer identified by whole exome next generation sequencing and bioinformatics analysis. PLoS ONE 2010;5:e15661.
25. Sanger Institute. COSMIC Database: Tissue overview for large intestine. Hinxton, England; 2012 [cited 2013 Jan 31]. Available from: http://www.sanger.ac.uk/perl/genetics/CGP/cosmic?action=byhist&s=4&hn=carcinoma&sn=large_intestine&ss=colon&ss=rectum&sh=adenocarcinoma.
26. Vaughn CP, Zobell SD, Furtado LV, Baker CL, Samowitz WS. Frequency of KRAS, BRAF, and NRAS mutations in colorectal cancer. Genes Chromosomes Cancer 2011;50:307–12.
27. Lievre A, Bachet JB, Boige V, Cayre A, Le Corre D, Buc E, et al. *KRAS* mutations as an independent prognostic factor in patients with advanced colorectal cancer treated with cetuximab. J Clin Oncol 2008;26:374–9.
28. Loupakis F, Ruzzo A, Cremolini C, Vincenzi B, Salvatore L, Santini D, et al. KRAS codon 61, 146 and BRAF mutations predict resistance to cetuximab plus irinotecan in KRAS codon 12 and 13 wild-type metastatic colorectal cancer. Br J Cancer 2009;101:715–21.
29. Peeters M, Douillard J-Y, Van Cutsem E, Siena S, Zhang K, Williams RT, et al. Mutant *KRAS* codon 12 and 13 alleles in patients with metastatic colorectal cancer: assessment as prognostic and predictive biomarkers of response to panitumumab. J Clin Oncol 2013;31:759–65.
30. Tejpar S, Celik I, Schlichting M, Sartorius U, Bokemeyer C, Van Cutsem E. Association of KRAS G13D tumor mutations with outcome in patients with metastatic colorectal cancer treated with first-line chemotherapy with or without cetuximab. J Clin Oncol 2012;30:3570–7.
31. De Roock W, Jonker DJ, Di Nicolantonio F, Sartore-Bianchi A, Tu D, Siena S, et al. Association of KRAS p.G13D mutation with outcome in patients with chemotherapy-refractory metastatic colorectal cancer treated with cetuximab. JAMA 2010;304:1812–20.
32. Irahara N, Baba Y, Nosho K, Shima K, Yan L, Dias-Santagata D, et al. NRAS mutations are rare in colorectal cancer. Diagn Mol Pathol 2010;19:157–63.
33. Fumagalli D, Gavin PG, Taniyama Y, Kim SI, Choi HJ, Paik S, et al. A rapid, sensitive, reproducible and cost-effective method for mutation profiling of colon cancer and metastatic lymph nodes. BMC Cancer 2010;10:101.
34. Richman SD, Seymour MT, Chambers P, Elliott F, Daly CL, Meade AM, et al. KRAS and BRAF mutations in advanced colorectal cancer are

Published OnlineFirst January 16, 2013; DOI: 10.1158/1078-0432.CCR-12-1913

associated with poor prognosis but do not preclude benefit from oxaliplatin or irinotecan: results from the MRC FOCUS trial. J Clin Oncol 2009;27:5931–7.

35. Farina-Sarasqueta A, van Lijnschoten G, Moerland E, Creemers GJ, Lemmens VE, Rutten HJ, et al. The BRAF V600E mutation is an independent prognostic factor for survival in stage II and stage III colon cancer patients. Ann Oncol 2010;21:2396–402.
36. Souglakos J, Philips J, Wang R, Marwah S, Silver M, Tzardi M, et al. Prognostic and predictive value of common mutations for treatment response and survival in patients with metastatic colorectal cancer. Br J Cancer 2009;101:465–72.
37. Saridaki Z, Papadatos-Pastos D, Tzardi M, Mavroudis D, Bairaktari E, Arvanity H, et al. BRAF mutations, microsatellite instability status and cyclin D1 expression predict metastatic colorectal patients' outcome. Br J Cancer 2010;102:1762–8.
38. Bokemeyer C, Bondarenko I, Hartmann JT, de Braud F, Schuch G, Zubel A, et al. Efficacy according to biomarker status of cetuximab plus FOLFOX-4 as first-line treatment for metastatic colorectal cancer: the OPUS study. Ann Oncol 2011;22:1535–46.
39. Sartore-Bianchi A, Martini M, Molinari F, Veronese S, Nichelatti M, Artale S, et al. PIK3CA mutations in colorectal cancer are associated with clinical resistance to EGFR-targeted monoclonal antibodies. Cancer Res 2009;69:1851–7.

Published OnlineFirst January 16, 2013; DOI: 10.1158/1078-0432.CCR-12-1913

Clinical Cancer Research

Massively Parallel Tumor Multigene Sequencing to Evaluate Response to Panitumumab in a Randomized Phase III Study of Metastatic Colorectal Cancer

Marc Peeters, Kelly S. Oliner, Alex Parker, et al.

Clin Cancer Res 2013;19:1902-1912. Published OnlineFirst January 16, 2013.