Published OnlineFirst September 4, 2015; DOI: 10.1158/1078-0432.CCR-15-0526



Clinical Cancer Research

# Analysis of *KRAS/NRAS* Mutations in a Phase III Study of Panitumumab with FOLFIRI Compared with FOLFIRI Alone as Second-line Treatment for Metastatic Colorectal Cancer

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## Abstract

**Purpose:** We evaluated the influence of *RAS* mutation status on the treatment effect of panitumumab in a prospective–retrospective analysis of a randomized, multicenter phase III study of panitumumab plus fluorouracil, leucovorin, and irinotecan (FOLFIRI) versus FOLFIRI alone as second-line therapy in patients with metastatic colorectal cancer (mCRC; ClinicalTrials.gov, NCT0039183).

**Experimental Design:** Outcomes were from the study's primary analysis. *RAS* mutations beyond *KRAS* exon 2 (*KRAS* exons 3, 4; *NRAS* exons 2, 3, 4; *BRAF* exon 15) were detected by bidirectional Sanger sequencing in wild-type *KRAS* exon 2 tumor specimens. Progression-free survival (PFS) and overall survival (OS) were coprimary endpoints.

**Results:** The *RAS* ascertainment rate was 85%; 18% of wild-type *KRAS* exon 2 tumors harbored other *RAS* mutations. For PFS and OS, the hazard ratio (HR) for panitumumab plus FOLFIRI versus FOLFIRI alone more strongly favored panitumumab in the wild-type *RAS* population than in the wild-type *KRAS* exon 2 population [PFS HR, 0.70 (95% confidence interval [CI], 0.54–0.91); $P = 0.007$ vs. 0.73 (95% CI, 0.59–0.90); $P = 0.004$; OS HR, 0.81 (95% CI, 0.63–1.03); $P = 0.08$ vs. 0.85 (95% CI, 0.70–1.04); $P = 0.12$]. Patients with *RAS* mutations were unlikely to benefit from panitumumab. Among *RAS* wild-type patients, the objective response rate was 41% in the panitumumab–FOLFIRI group versus 10% in the FOLFIRI group.

**Conclusions:** Patients with *RAS* mutations were unlikely to benefit from panitumumab–FOLFIRI and the benefit–risk of panitumumab–FOLFIRI was improved in the wild-type *RAS* population compared with the wild-type *KRAS* exon 2 population. These findings support *RAS* testing for patients with mCRC. *Clin Cancer Res; 21(24); 1–11. ©2015 AACR.*

*See related commentary by Salazar and Ciardiello, p. 5415*

## Introduction

The epidermal growth factor receptor (EGFR) is overexpressed in colorectal cancer (1), and plays an important role in cellular proliferation and metastasis in metastatic colorectal cancer (mCRC; ref. 2). The *RAS* family of small GTPases plays a central role in signaling downstream from the EGFR (3). Activating mutations in *RAS* can result in persistent signaling in the absence of ligand binding to the EGFR, and resistance to therapy with the anti-EGFR monoclonal antibodies panitumumab and cetuximab (3, 4). *KRAS* and *NRAS* activation result in different patterns of intracellular signaling, and mutations in *KRAS* and *NRAS* arise in different cellular contexts and are not functionally redundant (5). *KRAS* exon 2 mutations are an established predictive biomarker of

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

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Prior presentation: The results of this study have been presented in part at the 2014 ASCO Gastrointestinal Cancers Symposium (January 16–18, 2014; San Francisco, CA) and at the 2014 ASCO Annual Meeting (May 30–June 1, 2014; Chicago, IL).

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**doi:** 10.1158/1078-0432.CCR-15-0526

Published OnlineFirst September 4, 2015; DOI: 10.1158/1078-0432.CCR-15-0526



## Translational Relevance

In preclinical studies, identification of mutations in RAS enzymes that resulted in constitutive activation suggested that presence of these mutations may preclude response to anti–epidermal growth factor receptor (EGFR) therapy. Although studies have already demonstrated that commonly occurring *KRAS* exon 2 mutations in patients with metastatic colorectal cancer (mCRC) were associated with lack of response to anti-EGFR therapy, a large, prospective–retrospective analysis of a phase III study of panitumumab plus FOLFOX as first-line treatment in mCRC found that evaluation of a broader panel of *RAS* mutations (including mutations in *KRAS* exons 3 and 4, and *NRAS* exons 2, 3, and 4) better predicted patient outcomes. In this study, we found an improved benefit–risk profile (compared with *KRAS* exon 2 wild-type patients) for panitumumab plus FOLFIRI versus FOLFIRI alone among *RAS* wild-type patients and provide further support for *RAS* testing for patients with mCRC.

lack of response to anti-EGFR therapy in mCRC patients (6–10). These initial studies evaluated the most commonly occurring mutations in codons 12 and 13 of *KRAS*; predictive value of *KRAS* mutations beyond exon 2 and mutations in other *RAS* enzymes (such as *NRAS*) were not assessed (6–9). Based on large retrospective analyses (11) and results from hypothesis-generating studies using next-generation sequencing techniques (12, 13), analyses of studies evaluating anti-EGFR therapies as first-line therapy for mCRC demonstrated that additional activating mutations in *KRAS* exons 3 and 4 and *NRAS* exons 2, 3, and 4 predicted lack of response to panitumumab plus FOLFOX as first-line treatment (14, 15). However, there are limited data evaluating panitumumab in combination with irinotecan-based therapy by *RAS* status.

The primary analysis from the phase III, randomized, controlled 20050181 study demonstrated a significant improvement in median progression-free survival (PFS) and a trend toward improvement in overall survival (OS) with panitumumab plus fluorouracil, leucovorin, and irinotecan (FOLFIRI) compared with FOLFIRI alone in patients with *KRAS* exon 2 wild-type mCRC [PFS hazard ratio (HR), 0.73; 95% CI, 0.59–0.90; $P = 0.004$; OS HR, 0.85; 95% CI, 0.70–1.04; $P = 0.12$) but not in patients with mutated *KRAS* exon 2 mCRC (PFS HR, 0.85; 95% CI, 0.68–1.06; OS HR, 0.94; 95% CI, 0.76–1.15; ref. 8). This prospective–retrospective analysis demonstrated an improved benefit–risk profile of panitumumab plus FOLFIRI versus FOLFIRI alone among *RAS* wild-type patients enrolled in the 20050181 study.

## Materials and Methods

### Study design and eligibility

This prospective–retrospective analysis used data from an open-label, randomized, multicenter, phase III study comparing the efficacy of panitumumab plus FOLFIRI with FOLFIRI alone in patients with previously treated mCRC (ClinicalTrials.gov, NCT0039183). The primary analysis has been described previously (8). PFS and OS in the primary analysis population were the study's coprimary endpoints. Objective response rate (ORR) was a key secondary endpoint.

### Tumor specimens

For patients identified as wild-type KRAS exon 2 by an investigational-use-only assay in the primary study (Therascreen *KRAS* Mutation Kit; Qiagen and LightCycler; Roche Diagnostics), DNA for RAS analysis was extracted from banked formalin-fixed paraffin-embedded patient tumor specimens (DNA Extraction Mini Kit; Qiagen). Specimens containing <50% tumor area were macrodissected.

### Extended *RAS* analysis

Analysis of *KRAS* exon 3 (codons 59/61) and exon 4 (codons 117/146); *NRAS* exon 2 (codons 12/13), exon 3 (codons 59/61), and exon 4 (codons 117/146); and *BRAF* exon 15 (codon 600) was performed using gold-standard bidirectional Sanger sequencing and WAVE-based SURVEYOR Scan Kits (Transgenomic) was performed as previously described (14). Mutations and analysis methods were prespecified based on previous findings (14, 16–19). The central testing laboratory was blinded to treatment assignment and patient outcome.

### Assessments

Radiographic imaging (computed tomography/magnetic resonance imaging) was performed every 8 weeks throughout the study. Survival was monitored at 3-month intervals during long-term follow-up. Adverse events (AEs) occurring during the treatment phase and up to 30 days following the final dose of study drug were recorded and graded according to the NCI-CTCAE v3.0 with modifications for specified skin and nail toxicities (20). An independent data monitoring committee oversaw the safety analysis.

### Statistical analysis

The statistical analysis plan for this *RAS* analysis was developed after the *KRAS* exon 2 analysis was unblinded but before the *RAS* and *BRAF* mutational analysis was done. Clinical outcomes were from the primary analysis.

The primary objective was to evaluate by *RAS* and *BRAF* status the treatment effect of panitumumab plus FOLFIRI versus FOLFIRI alone on PFS and OS in the primary analysis population. For the purposes of this analysis, patients were characterized as having *RAS* mutations if analysis identified any predefined activating mutation in *KRAS* or *NRAS*. Similarly, patients were characterized as having *RAS* or *BRAF* mutations if any predefined *RAS* or *BRAF* mutation was detected.

Hypothesis testing was exploratory and similar to that employed in extended *RAS* analysis of the PRIME study (14). A sequential testing scheme evaluated the treatment effect of panitumumab plus FOLFIRI versus FOLFIRI alone on PFS followed by a test of the treatment effects on OS among patients with wild-type *RAS* and wild-type *RAS* and *BRAF* (5% significance level). Effects of panitumumab on PFS and OS within each biomarker group were evaluated using log-rank tests stratified by the randomization factors. The magnitude of the panitumumab treatment effect on OS and PFS was calculated using Cox proportional hazards models stratified by the randomization factors. All randomized patients within each biomarker subgroup were included. Tumor response was evaluated per RECIST by blinded independent central radiology review for patients with $\geq$1 unidimensionally measurable lesion (21). Responses were confirmed $\geq$28 days after the criteria for response were first met. Analyses of early tumor response only included those patients with available baseline and

Published OnlineFirst September 4, 2015; DOI: 10.1158/1078-0432.CCR-15-0526



week 8 measurements. Differences in early tumor response between groups were evaluated using a Fisher exact test. For patients with reductions from baseline in tumor size, median depth of response was calculated as percentage change from baseline to nadir. For patients with tumor growth or no change in tumor dimensions (i.e., with no recorded tumor shrinkage), depth of response was defined as percentage change from baseline to progression or as missing if the patient did not have progression. Differences in depth of response were evaluated using a Wilcoxon test.

## Results

### Patients

Among the 1,186 patients randomized, *RAS* status was ascertained in 1,014 (85%) patients (Supplementary Fig. S1; Supplementary Table S1). Among these patients, 421 (42%) had wild-type *RAS* tumors (panitumumab + FOLFIRI, $n = 208$; FOLFIRI alone, $n = 213$) and 593 (58%) had mutated *RAS* tumors (panitumumab + FOLFIRI, $n = 299$; FOLFIRI alone, $n = 294$). Among the 597 patients evaluated as having wild-type *KRAS* exon 2 tumors in the primary analysis, 107 (18%; panitumumab + FOLFIRI, $n = 61$; FOLFIRI alone, $n = 46$) were found to have other *RAS* mutations (*KRAS* exons 3/4 or *NRAS*) in this study. Among patients with wild-type *RAS*, 376/421 (89%) had wild-type *BRAF* and 45/421 (11%) had mutant *BRAF*. Of the 1,186 randomized patients, 638 (54%) had mutant *RAS* or mutant *BRAF*.

Baseline clinical/demographic characteristics were similar between treatment arms and between patients with wild-type and mutated *RAS*, and were similar to the baseline demographics in the wild-type *KRAS* exon 2 population as previously reported (Table 1; ref. 8).

### Efficacy outcomes by tumor *RAS* mutation status

For PFS, the HR for panitumumab plus FOLFIRI versus FOLFIRI alone was 0.73 (95% CI, 0.59–0.90; $P = 0.004$; Fig. 1A) in patients with wild-type *KRAS* exon 2 compared with 0.70 (95% CI, 0.54–0.91; $P = 0.007$; Fig. 1B) in patients with wild-type *RAS*. Estimated median PFS was longest in the *RAS* wild-type panitumumab plus FOLFIRI group. For OS, the HR for panitumumab plus FOLFIRI versus FOLFIRI alone more strongly favored panitumumab in the extended wild-type *RAS* population than in the wild-type *KRAS* exon 2 population (HR, 0.81; [95% CI, 0.63–1.03]; $P = 0.08$ vs. HR, 0.85; [95% CI, 0.70–1.04]; $P = 0.12$; Fig. 1C and D). Again, estimated median OS was longest in the *RAS* wild-type panitumumab plus FOLFIRI group. Sensitivity analyses using Branson and Whitehead models (22) and Law methods (23), did not provide evidence of an influence of postprogression anti-EGFR therapy on OS time.

Patients with *RAS* mutations did not derive clinical benefit from panitumumab plus FOLFIRI and there was no evidence that outcomes were worse or of a negative interaction between the administered agents. Among patients with wild-type *KRAS* exon 2 but with other *RAS* mutations, the HR for PFS for panitumumab plus FOLFIRI versus FOLFIRI alone was 0.89 (95% CI, 0.56–1.42; $P = 0.63$; Fig. 2A). Among patients with any *RAS* mutation, the HR for PFS for panitumumab plus FOLFIRI versus FOLFIRI alone was 0.86 (95% CI, 0.71–1.05; $P = 0.14$; Fig. 2B). Findings were similar for OS (Fig. 2C and D) in patients with any *RAS* mutation. Among patients with mutated *KRAS* exon 2, the HR for PFS for panitumumab plus FOLFIRI versus FOLFIRI was 0.85 (95% CI, 0.68–1.06); for OS the HR was 0.94 (95% CI, 0.76–1.15; Fig. 3A).

Quantitative interaction tests for the negative predictive value of *RAS* mutations beyond those in *KRAS* exon 2 on panitumumab treatment effect were not statistically significant (PFS, $P = 0.37$; OS, $P = 0.93$).

### Efficacy outcomes by tumor *BRAF* mutation status

*BRAF* mutation status was not predictive of benefit with panitumumab. Among patients with wild-type *RAS* and wild-type *BRAF* ($n = 376$), the HR for panitumumab plus FOLFIRI versus FOLFIRI alone was 0.68 (95% CI, 0.51–0.90; 6.9 months vs. 5.5 months; $P = 0.006$); similarly, in patients with wild-type *RAS* and mutated *BRAF* ($n = 45$), the HR for panitumumab plus FOLFIRI versus FOLFIRI alone was 0.69 (95% CI, 0.32–1.49; 2.5 months vs. 1.8 months; $P = 0.34$; Fig. 3A). Similar results were observed for OS: the HR among patients with wild-type *RAS* and wild-type *BRAF* was 0.83

**Table 1.** Baseline demographic and clinical characteristics by *RAS* status

| | Wild-type *RAS* | | Mutated *RAS* | |
|---|---|---|---|---|
| **Characteristic** | **Panitumumab + FOLFIRI ($N = 208$)** | **FOLFIRI alone ($N = 213$)** | **Panitumumab + FOLFIRI ($N = 299$)** | **FOLFIRI alone ($N = 294$)** |
| Men | 136 (65) | 140 (66) | 165 (55) | 177 (60) |
| Median (range) age, y | 60 (28–81) | 60 (33–85) | 61 (29–84) | 64 (29–86) |
| Race, white | 203 (98) | 202 (95) | 284 (95) | 283 (96) |
| ECOG performance status 0–1 | 196 (94) | 198 (93) | 284 (95) | 275 (94) |
| Region | | | | |
| Western EU, Canada, Australia | 136 (65) | 139 (65) | 184 (62) | 182 (62) |
| Rest of the world | 72 (35) | 74 (35) | 115 (38) | 112 (38) |
| Primary tumor type | | | | |
| Colon | 119 (57) | 148 (69) | 201 (67) | 186 (63) |
| Rectal | 89 (43) | 65 (31) | 98 (33) | 108 (37) |
| Sites of metastatic disease | | | | |
| Liver only | 37 (18) | 49 (23) | 46 (15) | 40 (14) |
| Liver plus other | 140 (67) | 134 (63) | 213 (71) | 204 (69) |
| Subsequent therapies | | | | |
| Bevacizumab | 21 (10) | 25 (12) | 39 (13) | 30 (10) |
| EGFR mAb | 21 (10) | 68 (32) | 24 (8) | 91 (31) |
| Oxaliplatin, irinotecan, or FU | 93 (45) | 107 (50) | 138 (46) | 151 (51) |

NOTE: Data are presented as *n* (%) unless otherwise noted.

Abbreviations: ECOG, Eastern Cooperative Oncology Group; EGFR, epidermal growth factor receptor; EU, European Union; FOLFIRI, fluorouracil, leucovorin, and irinotecan; FU, fluorouracil; mAb, monoclonal antibody.

Published OnlineFirst September 4, 2015; DOI: 10.1158/1078-0432.CCR-15-0526



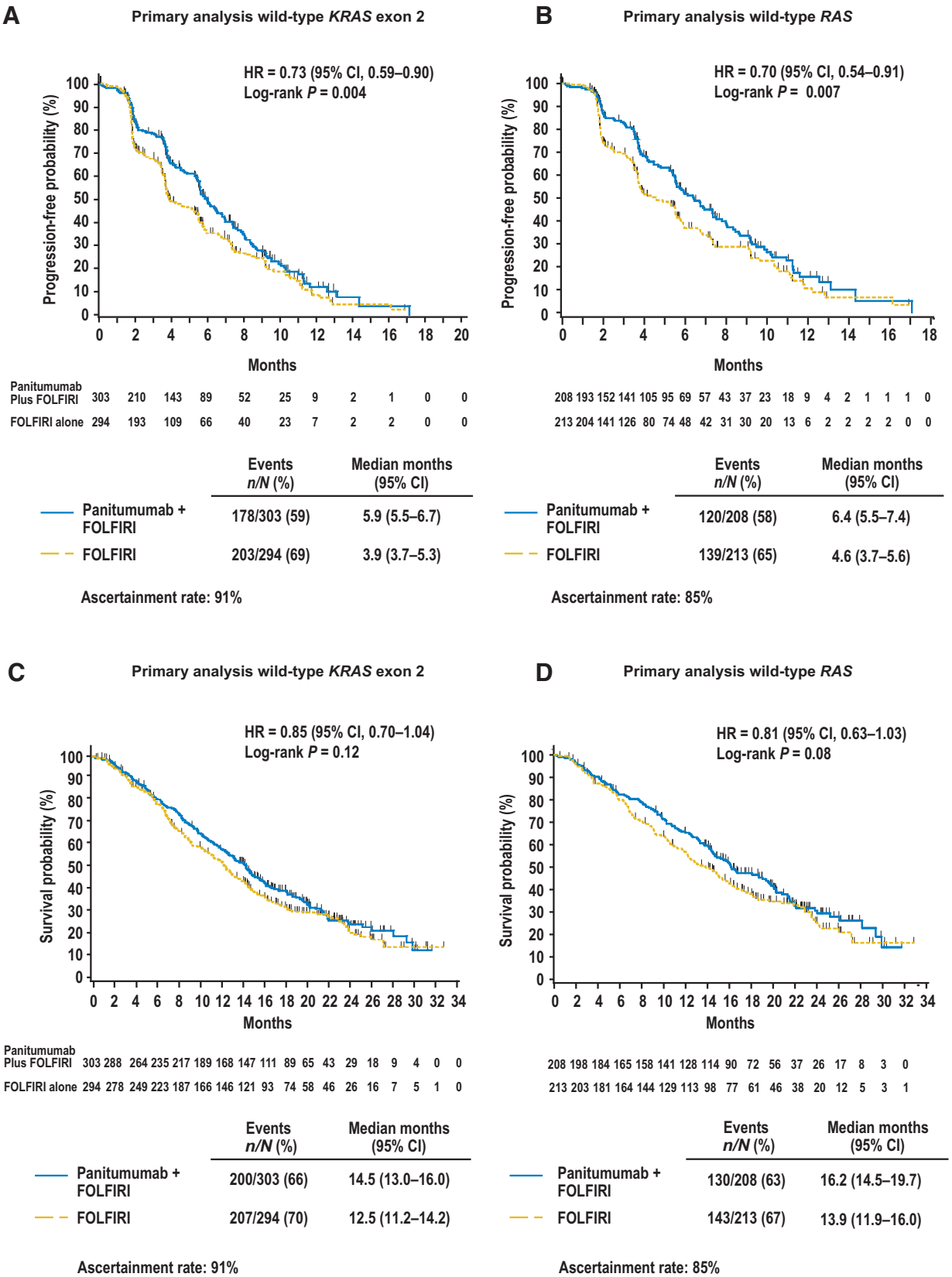


**Figure 1.**
PFS and OS among patients with wild-type *KRAS* exon 2 and among patients with wild-type extended *RAS*. FOLFIRI, fluorouracil, leucovorin, and irinotecan; HR, hazard ratio; OS, overall survival; PFS, progression-free survival; WT, wild-type.

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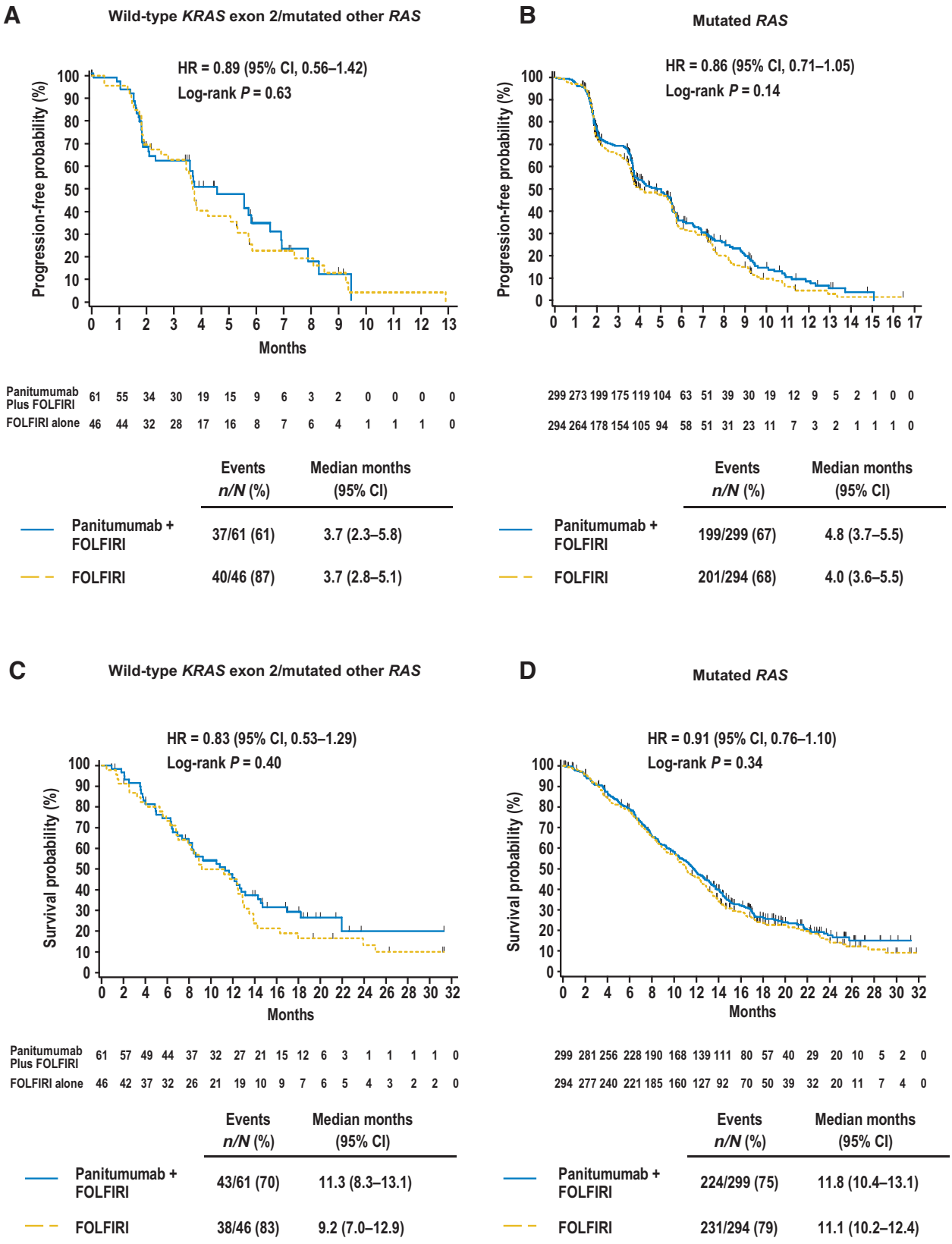


**Figure 2.**
PFS and OS among patients with wild-type *KRAS* exon 2 and another *RAS* mutation and among patients with any *RAS* mutation. FOLFIRI, fluorouracil, leucovorin, and irinotecan; HR, hazard ratio; MT, mutated; OS, overall survival; PFS, progression-free survival; WT, wild-type.

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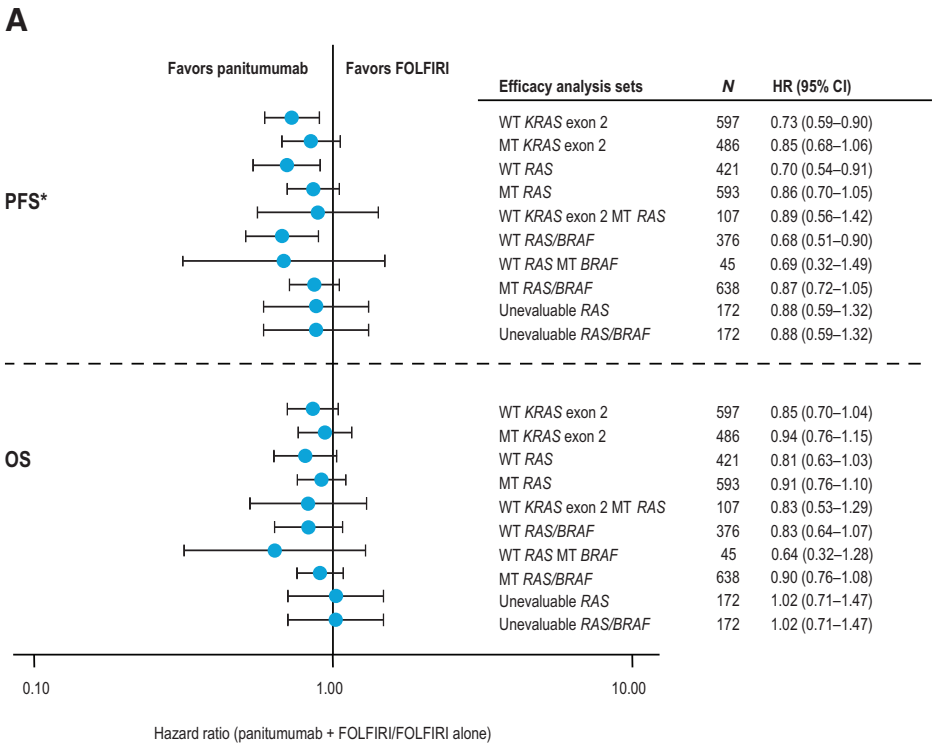



**Figure 3.**
A, Hazard ratios for PFS and OS for panitumumab plus FOLFIRI versus FOLFIRI alone by *KRAS* and *RAS* mutation status. B, Hazard ratios for PFS and OS for wild-type and mutated *BRAF*. *PFS by central assessment. FOLFIRI, fluorouracil, leucovorin, and irinotecan; HR, hazard ratio; MT, mutated; OS, overall survival; PFS, progression-free survival; Pmab, panitumumab; WT, wild-type.

(95% CI, 0.64–1.07; 18.7 months vs. 15.4 months; $P = 0.15$) and the HR among patients with wild-type *RAS* and mutated *BRAF* was 0.64 (95% CI, 0.32–1.28; 4.7 months vs. 5.7 months; $P = 0.20$). Irrespective of assigned treatment, the HR for PFS favored patients with wild-type *BRAF* versus those with mutated *BRAF* (HR, 0.28; 95% CI, 0.20–0.40; $n = 421$). For OS, the HR was 0.25 (95% CI, 0.18–0.36). The presence of a *BRAF* mutation was associated with poorer prognosis (Fig. 3B).

### Tumor response

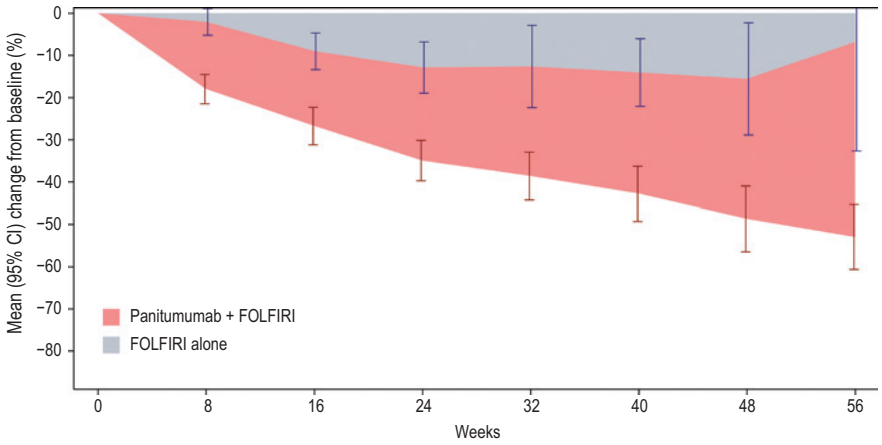
In *KRAS* exon 2 wild-type patients, the ORR was 35% in the panitumumab plus FOLFIRI group versus 10% in the FOLFIRI alone group, whereas in patients with wild-type *RAS*, the ORR was 41% in the panitumumab plus FOLFIRI group and 10% in the FOLFIRI alone group (Fig. 4 and Supplementary Table S2). ORR was similar for panitumumab plus FOLFIRI and FOLFIRI alone among patients with any *RAS* mutation (15% vs. 13%; Supplementary Table S1) and for patients with mutated *KRAS* exon 2 (13% vs. 14%, respectively). Median duration of response among *RAS* wild-type patients was 9.3 months for panitumumab plus FOLFIRI versus 7.7 months for FOLFIRI alone (Fig. 4).

Exploratory response assessments were performed to describe the timing and magnitude of response. For patients with wild-type *RAS*, mean percentage change from baseline in the sum of longest diameter of target lesions was markedly greater among patients who received panitumumab (Fig. 4). Depth of response (assessed by median percentage tumor shrinkage) was greater with panitumumab plus FOLFIRI versus FOLFIRI alone (37% vs. 10%; $P < 0.0001$; Fig. 4). Similarly, a greater proportion of wild-type *RAS* patients receiving panitumumab plus FOLFIRI had a $\geq$30% change in sum of longest diameter of target lesions within the

Published OnlineFirst September 4, 2015; DOI: 10.1158/1078-0432.CCR-15-0526



**A**




| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Panitumumab + FOLFIRI | 203 | 181 | 143 | 112 | 77 | 63 | 42 | 28 |
| FOLFIRI alone | 204 | 180 | 126 | 88 | 53 | 33 | 20 | 12 |

**B**

| | Panitumumab + FOLFIRI | | FOLFIRI alone | |
|---|---|---|---|---|
| Wild-type *KRAS* exon 2, *n* | 297 | | 285 | |
| Objective response rate, *n* (%) | 105 (35) | | 28 (10) | |
| 95% CI | 30–41 | | 7–14 | |
| Median duration of response, mo (95% CI) | 7.6 (6.7–9.4) | | 6.6 (5.7–10.4) | |
| Wild-type *RAS*, *n* | 204 | | 207 | |
| Objective response rate, *n* (%) | 83 (41) | | 21 (10) | |
| 95% CI | 34–48 | | 6–15 | |
| Median duration of response, mo (95% CI) | 7.7 (6.7–9.9) | | 9.3 (6.1–12.8) | |
| Depth of response*, *n* | 177 | | 172 | |
| Median (interquartile range) tumor shrinkage, % | 37 (13–56) | | 10 (–5 to 26) | |
| *P* | <0.0001 | | | |
| Tumor shrinkage within first 8 weeks for wild-type *RAS* patients† | <30% | ≥30% | <30% | ≥30% |
| Patients, *n* (%) | 114 (63) | 67 (37) | 168 (93) | 12 (7) |
| *P* | <0.0001 | | | |

**Figure 4.**
A, Mean (95% CI) percentage change from baseline in sum of longest diameters for patients with wild-type *RAS*. B, Objective response rate, duration of response, depth of response, and association between early tumor shrinkage and PFS/OS in patients with wild-type *RAS*. Duration of response was defined as the time from first confirmed objective response to disease progression per RECIST. * Percent tumor shrinkage from baseline; positive values indicate reduction in tumor size, whereas negative values indicate an increase in tumor size; *P* value for difference between arms determined by Wilcoxon test. † Evaluated for patients with baseline and week 8 tumor measurements; *P* value for difference between groups within the contingency table determined by Fisher's exact test. FOLFIRI, fluorouracil, leucovorin, and irinotecan.

first 8 weeks of treatment compared with those receiving FOLFIRI alone (37% vs. 7%; $P < 0.0001$). Early tumor response and depth of response outcomes were more favorable in panitumumab-treated wild-type *RAS* patients than panitumumab-treated wild-type *KRAS* exon 2 patients (Supplementary Table S3).

### Adverse events

The types, incidence rates, and severity of AEs were similar in patients with wild-type *RAS* and mutated *RAS* in the panitumumab plus FOLFIRI arm (Table 2). Additionally, the nature and frequency of incidence of AEs was similar to that previously reported for the wild-type *KRAS* exon 2 population (8). The most frequently occurring AEs reported among all patients were diarrhea, rash, nausea, fatigue, and neutropenia. The incidence of hypomagnesemia and skin toxicities were higher with panitumumab plus FOLFIRI compared with FOLFIRI alone (Table 2). In patients with wild-type *RAS*, 24% in the panitumumab plus FOLFIRI group and 12% in the FOLFIRI alone group had AEs leading to discontinuation.

## Discussion

Routine *KRAS* exon 2 mutation testing has allowed for identification of patients with mCRC more likely to derive benefit from panitumumab. However, a substantial proportion of patients with wild-type *KRAS* exon 2 mCRC do not respond to panitumumab therapy, and there is potential for further refinement of patient selection. Results from this prospective–retrospective analysis provide support for use of this regimen in patients with *RAS* wild-type mCRC. We found improvements in the treatment effect for panitumumab plus FOLFIRI versus FOLFIRI alone for both PFS and OS in the wild-type *RAS* mCRC group compared with the wild-type *KRAS* exon 2 mCRC group. Conversely, patients with *RAS* mutations beyond *KRAS* exon 2 or with any RAS mutation were unlikely to benefit from addition of panitumumab to FOLFIRI. Although there was a trend toward longer OS among wild-type *KRAS* exon 2/mutated other *RAS* patients (11.3 months vs. 9.2 months), PFS was similar (3.7 months in both groups), and exclusion of wild-type *RAS* patients did not alter ORR. Importantly, there was no evidence of worsening of OS or PFS with panitumumab treatment in the mutated *RAS* group. High *RAS* ascertainment (85%) was a strength of the study, ensuring the *RAS*-evaluable population was likely representative of the overall population and allowing for a robust estimate of the proportion (18%) of patients with wild-type *KRAS* exon 2 tumors harboring other *RAS* mutations.

The totality of available evidence supports routine use of *RAS* analysis. For panitumumab, our results in the second-line setting are consistent with those from a previous prospective–retrospective *RAS* analysis of the PRIME study (which evaluated

Published OnlineFirst September 4, 2015; DOI: 10.1158/1078-0432.CCR-15-0526



**Table 2.** Summary of adverse events by *RAS* status

| Adverse event, *n* (%) | Wild-type *RAS* | | Mutated *RAS* | |
|---|---|---|---|---|
| | Panitumumab + FOLFIRI (*N* = 207) | FOLFIRI alone (*N* = 213) | Panitumumab + FOLFIRI (*N* = 298) | FOLFIRI alone (*N* = 292) |
| Any AE | 207 (100) | 211 (99) | 296 (99) | 281 (96) |
| Worst grade of 3 | 114 (55) | 78 (37) | 137 (46) | 100 (34) |
| Worst grade of 4 | 41 (20) | 35 (16) | 50 (17) | 44 (15) |
| Worst grade of 5 | 8 (4) | 13 (6) | 21 (7) | 17 (6) |
| Serious AE | 94 (45) | 67 (31) | 110 (37) | 90 (31) |
| AEs occurring in ≥20% of patients in either treatment arm | | | | |
| Diarrhea | 142 (69) | 122 (57) | 181 (61) | 167 (57) |
| Rash | 111 (54) | 17 (8) | 167 (56) | 16 (5) |
| Nausea | 104 (50) | 106 (50) | 142 (48) | 129 (44) |
| Fatigue | 81 (39) | 69 (32) | 102 (34) | 104 (36) |
| Neutropenia | 79 (38) | 87 (41) | 95 (32) | 97 (33) |
| Hypomagnesemia | 61 (29) | 5 (2) | 47 (16) | 6 (2) |
| Vomiting | 59 (29) | 62 (29) | 82 (28) | 84 (29) |
| Dermatitis acneiform | 57 (28) | 2 (1) | 71 (24) | 1 (<1) |
| Anorexia | 56 (27) | 34 (16) | 71 (24) | 49 (17) |
| Abdominal pain | 54 (26) | 41 (19) | 50 (17) | 61 (21) |
| Stomatitis | 54 (26) | 28 (13) | 62 (21) | 38 (13) |
| Alopecia | 51 (25) | 48 (23) | 54 (18) | 78 (27) |
| Constipation | 49 (24) | 46 (22) | 75 (25) | 65 (22) |
| Dry skin | 46 (22) | 11 (5) | 65 (22) | 10 (3) |
| Paronychia | 46 (22) | 0 | 40 (13) | 2 (1) |
| Pruritus | 42 (20) | 9 (4) | 47 (16) | 8 (3) |
| Pyrexia | 41 (20) | 42 (20) | 61 (20) | 49 (17) |
| Skin fissures | 41 (20) | 1 (<1) | 41 (14) | 2 (1) |
| Mucosal inflammation | 39 (19) | 30 (14) | 67 (22) | 36 (12) |
| Anemia | 37 (18) | 49 (23) | 36 (12) | 45 (15) |

Abbreviations: AE, adverse event; FOLFIRI, fluorouracil, leucovorin, and irinotecan.

panitumumab plus FOLFOX4 vs. FOLFOX4 as first-line therapy; ref. 14), a prospective *RAS* analysis of the PEAK study (which evaluated panitumumab or bevacizumab plus FOLFOX as first-line therapy; ref. 15), and the original hypothesis-generating analysis of the 408 study (which evaluated panitumumab monotherapy in patients with chemotherapy-refractory disease; refs. 12, 13). The results are also consistent with analysis of two smaller studies that showed improvements in response rate with *RAS* analysis among patients with chemotherapy-refractory disease receiving panitumumab plus irinotecan (24) or liver-limited disease receiving neoadjuvant panitumumab plus FOLFOX/FOLFIRI (25), respectively. Similar results have also been reported in cetuximab studies. Recent retrospective analyses of studies evaluating first-line FOLFIRI ± cetuximab [CRYSTAL (26), FIRE-3 (27), and CAPRI-GOIM (28)] or FOLFOX ± cetuximab [OPUS (29)] demonstrated potential predictive value for *RAS* analysis. In the CALGB/SWOG-80405 study of first-line FOLFOX/FOLFIRI plus cetuximab or bevacizumab, there appeared to be little if any improvement in the OS or PFS HR in patients with wild-type *RAS* versus patients with wild-type *KRAS* exon 2 (30). Notably, *RAS* ascertainment was somewhat lower in the cetuximab studies particularly CALGB/SWOG-80405 (CRYSTAL, 69%; OPUS, 75%; FIRE-3, 69%; CALGB/SWOG-80405, 55%; and CAPRI-GOIM, 54%). The distribution of additional *RAS* mutations by chemotherapy backbone in CALGB/SWOG-80405 and interaction testing have yet to be reported. This and the low *RAS* ascertainment limit interpretation of the results. Overall, results from panitumumab and cetuximab studies indicate that patients with *RAS* mutant mCRC are unlikely to benefit from anti-EGFR therapy irrespective of chemotherapy or line of therapy.

These results strongly support routine *RAS* analysis in mCRC. Testing for *RAS* mutations beyond *KRAS* exon 2 better predicts response to treatment and improves patient selection, thereby sparing patients who are unlikely to respond potential toxicities associated with anti-EGFR therapy. Rates of *RAS* mutation beyond *KRAS* exon 2 from 10% to 26% (14, 15, 29, 31–33) have been reported in recent studies using technologies including pyrosequencing and BEAMing. NCCN (34, 35), ESMO (36), and the European Society of Pathology (35) recommend *KRAS/NRAS* genotyping for patients with mCRC, and the Association of Clinical Pathologists Molecular Pathology and Diagnostics Group has issued a guidance document describing *RAS* testing requirements in the United Kingdom (37). Consistency and validation of testing techniques and appropriate timing of their use will be important for clinical application of *RAS* analysis.

Patients with *BRAF* mutations had shorter estimated median PFS and OS than *BRAF* wild-type patients, consistent with previous findings (11, 14, 33). This difference in prognosis was independent of patients' *RAS* mutation status or panitumumab treatment. In this study, *BRAF* mutations did not have clear predictive value and the results do not provide support for *BRAF* mutation testing to guide anti-EGFR therapy. However, the prognostic information might guide other clinical decisions. To improve outcomes for these patients, recent studies have evaluated feasibility of treatment with anti-EGFR antibodies and other targeted agents (38, 39).

The 41% ORR in the wild-type *RAS* panitumumab group represents one of the highest rates reported in the second-line setting, and should be considered when selecting second-line therapy. Evaluation of other measures of tumor response may inform clinical decision-making (although such measures require further prospective confirmation; ref. 40). Depth of tumor response was significantly greater and likelihood of achieving a ≥30% reduction in tumor dimensions within 8 weeks of treatment was significantly higher in panitumumab patients. Both outcomes were improved in *RAS* wild-type patients versus *KRAS*

Published OnlineFirst September 4, 2015; DOI: 10.1158/1078-0432.CCR-15-0526



exon 2 wild-type patients. Studies with cetuximab have reported associations between early tumor shrinkage (41) and depth of tumor response (42) and survival. Whether similar associations between these measures and survival occur with panitumumab remains to be evaluated.

Selecting patients using extended *RAS* analysis did not alter the safety profile of panitumumab. Consistent with previous studies, toxicities occurring more frequently among panitumumab-treated patients included skin/nail toxicities and hypomagnesemia. There was no evidence of negative interactions between panitumumab and irinotecan in patients with *RAS* mutations, consistent with the CRYSTAL (26) and COIN (43) studies. Poorer OS among *NRAS*-mutant patients receiving panitumumab plus irinotecan versus irinotecan alone was reported in the PICCOLO study, but these outcomes may have been influenced by the Q3W treatment schedule used (44). These data were also in contrast to the results seen in the PRIME (14) and OPUS (29) studies, in which outcomes were worse with panitumumab or cetuximab in combination with oxaliplatin-containing therapy (FOLFOX) in patients with *RAS*-mutant tumors, compared with FOLFOX alone.

Key limitations of this study were that *RAS* analysis was exploratory (not defined in the original study protocol) and that results from the *KRAS* exon 2 analysis were known before this analysis was initiated. Consequently, the potential for bias exists. However, the biomarker hypothesis was developed before the mutational analysis was available and was limited to *RAS*/*BRAF*. Moreover, tumor specimens and clinical outcome data were derived from a large randomized phase III study, and the high rate of *RAS* ascertainment limited the potential for ascertainment bias. *RAS* was evaluated using robust, widely available assay procedures. The small number of patients in some groups limits our ability to draw conclusions regarding outcomes. A variety of confounding factors (e.g., postprogression therapy) might have limited our ability to detect improvement in OS.

Results from this study provide compelling evidence for panitumumab plus irinotecan–based therapy as an important second-line therapy for *RAS* wild-type patients supported by phase III evidence. Exclusion of patients with *RAS* mutations improved the benefit–risk profile of panitumumab plus FOLFIRI in this setting. The totality of evidence supporting *RAS* analysis supports use of these analytical techniques in upfront testing. A recent meta-analysis of nine panitumumab and cetuximab studies found improvements in outcomes with extended *RAS* analysis (45). Patient-level meta-analyses across randomized studies [including this study, PRIME (14), PEAK (15), the 408 study (12, 13), and ongoing 0007 study (ClinicalTrials.gov, NCT01412957), and studies in which patients received bevacizumab] may provide increased statistical power for evaluation of *RAS* analysis.

## Disclosure of Potential Conflicts of Interest

M. Peeters is a consultant for Amgen, Bayer, Celgene, Eli Lilly, Merck, Merrimack, Roche, Sanofi, Serono, and Taiho; reports receiving commercial research grants from Aduro, Amgen, Boehringer Ingelheim, Bristol-Myers Squibb, Celgene, Dekkun, Eli Lilly, Fresenius Biotech, GlaxoSmithKline, Halozyme, Merck, Millennium, Novartis, Pfizer, Roche, and Sereno; and speakers bureau honoraria from Amgen, Bayer, Biocompatibles, Merck, Novartis, Pfizer, Roche, Sanofi, and Serono. T.J. Price and A. Cervantes are consultant/advisory board members for Amgen. A.F. Sobrero reports receiving speakers bureau honoraria from Amgen, Bayer, Merck, and Roche and is a consultant/advisory board member for Amgen and Merck. M. Ducreux reports receiving a commercial research grant from Roche; other commercial research support from Pfizer; speakers bureau honoraria from Amgen, Bayer, Merck Serono, Novartis, and Roche; and is a consultant/advisory board member for Amgen, Eli Lilly, Merck Sereno, and Roche. T. Andre reports receiving speakers bureau honoraria from and is a consultant/advisory board member for Amgen. E. Chan is a consultant/advisory board member for Amgen, Bayer, Castle Biosciences, Eli Lilly, Genentech, Merrimack, and Taiho. F. Lordick reports receiving commercial research grants from Fresenius Biotech and GlaxoSmithKline and is a consultant/advisory board member for Bristol-Myers Squibb, Ganymed, and Nordic. C.J.A. Punt is a consultant/advisory board member for Amgen and Merck Serono. T.E. Ciuleanu is a consultant/advisory board member for Amgen, Merck, and Roche. E.V. Cutsem reports receiving a commercial research grant from Amgen. J.-H. Terwey and A.S. Jung have ownership interest in Amgen. No potential conflicts of interest were disclosed by the other authors.

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## Acknowledgments

The authors thank Mark Ekdahl (Amgen Inc.) for operational assistance and Ali Hassan, PhD, and Anny Wu, PharmD (both Complete Healthcare Communications, Inc.), for medical writing assistance funded by Amgen Inc.

## Grant Support

This work was supported by Amgen Inc.

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Received March 4, 2015; revised May 28, 2015; accepted June 25, 2015; published OnlineFirst September 4, 2015.

## References

1. Vectibix® (panitumumab). Full prescribing information. Thousand Oaks, CA: Amgen Inc.; 2014.
2. Radinsky R, Risin S, Fan D, Dong Z, Bielenberg D, Bucana CD, et al. Level and function of epidermal growth factor receptor predict the metastatic potential of human colon carcinoma cells. Clin Cancer Res 1995;1:19–31.
3. van Krieken JH, Jung A, Kirchner T, Carneiro F, Seruca R, Bosman FT, et al. KRAS mutation testing for predicting response to anti-EGFR therapy for

Published OnlineFirst September 4, 2015; DOI: 10.1158/1078-0432.CCR-15-0526



colorectal carcinoma: proposal for an European quality assurance program. Virchows Arch 2008;453:417–31.
4. Young A, Lou D, McCormick F. Oncogenic and wild-type Ras play divergent roles in the regulation of mitogen-activated protein kinase signaling. Cancer Discov 2013;3:112–23.
5. Wang Y, Velho S, Vakiani E, Peng S, Bass AJ, Chu GC, et al. Mutant N-RAS protects colorectal cancer cells from stress-induced apoptosis and contributes to cancer development and progression. Cancer Discov 2013;3:294–307.
6. 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.
7. 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.
8. 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.
9. 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.
10. Van Cutsem E, Kohne CH, Lang I, Folprecht G, Nowacki MP, Cascinu S, et al. Cetuximab plus irinotecan, fluorouracil, and leucovorin as first-line treatment for metastatic colorectal cancer: updated analysis of overall survival according to tumor KRAS and BRAF mutation status. J Clin Oncol 2011;29:2011–9.
11. 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.
12. Patterson SD, Peeters M, Siena S, Van Cutsem E, Humblet Y, Laethem JV, et al. Comprehensive analysis of KRAS and NRAS mutations as predictive biomarkers for single agent panitumumab (pmab) response in a randomized, phase III metastatic colorectal cancer (mCRC) study (20020408). J Clin Oncol 2013;31:abstract 3617.
13. Peeters M, Oliner KS, Parker A, Siena S, Van Cutsem E, Huang J, et al. Massively parallel tumor multigene sequencing to evaluate response to panitumumab in a randomized phase III study of metastatic colorectal cancer. Clin Cancer Res 2013;19:1902–12.
14. Douillard JY, Oliner KS, Siena S, Tabernero J, Burkes R, Barugel M, et al. Panitumumab-FOLFOX4 treatment and RAS mutations in colorectal cancer. N Engl J Med 2013;369:1023–34.
15. Schwartzberg LS, Rivera F, Karthaus M, Fasola G, Canon JL, Hecht JR, et al. PEAK: a randomized, multicenter phase II study of panitumumab plus modified fluorouracil, leucovorin, and oxaliplatin (mFOLFOX6) or bevacizumab plus mFOLFOX6 in patients with previously untreated, unresectable, wild-type KRAS exon 2 metastatic colorectal cancer. J Clin Oncol 2014;32:2240–7.
16. 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.
17. 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.
18. Janakiraman M, Vakiani E, Zeng Z, Pratilas CA, Taylor BS, Chitale D, et al. Genomic and biological characterization of exon 4 KRAS mutations in human cancer. Cancer Res 2010;70:5901–11.
19. Edkins S, O'Meara S, Parker A, Stevens C, Reis M, Jones S, et al. Recurrent KRAS codon 146 mutations in human colorectal cancer. Cancer Biol Ther 2006;5:928–32.
20. Cancer Therapy Evaluation Program. Common Terminology Criteria for Adverse Events v3.0 (CTCAE). [cited Jun 2, 2014]. Available from: http://ctep.cancer.gov/protocoldevelopment/electronic_applications/docs/ctcaev3.pdf.
21. 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.
22. Branson M, Whitehead J. Estimating a treatment effect in survival studies in which patients switch treatment. Stat Med 2002;21:2449–63.
23. Law MG, Kaldor JM. Survival analyses of randomized clinical trials adjusted for patients who switch treatments. Stat Med 1996;15:2069–76.
24. Andre T, Blons H, Mabro M, Chibaudel B, Bachet JB, Tournigand C, et al. Panitumumab combined with irinotecan for patients with KRAS wild-type metastatic colorectal cancer refractory to standard chemotherapy: a GERCOR efficacy, tolerance, and translational molecular study. Ann Oncol 2013;24:412–9.
25. Abad A, Massuti B, Gravalos C, Pilar E, Guillen C, Manzano JL, et al. Phase II trial of panitumumab plus FOLFOX4 or FOLFIRI in subjects with KRAS wild-type colorectal cancer and liver-limited disease: The PLANET study. J Clin Oncol 2014;32:abstract 3650.
26. Van Cutsem E, Lenz HJ, Kohne CH, Heinemann V, Tejpar S, Melezinek I, et al. Fluorouracil, leucovorin, and irinotecan plus cetuximab treatment and RAS mutations in colorectal cancer. J Clin Oncol 2015;33:692–700.
27. Heinemann V, von Weikersthal LF, Decker T, Kiani A, Vehling-Kaiser U, Al-Batran SE, et al. FOLFIRI plus cetuximab versus FOLFIRI plus bevacizumab as first-line treatment for patients with metastatic colorectal cancer (FIRE-3): a randomised, open-label, phase 3 trial. Lancet Oncol 2014;15:1065–75.
28. Ciardiello F, Normanno N, Maiello E, Martinelli E, Troiani T, Pisconti S, et al. Clinical activity of FOLFIRI plus cetuximab according to extended gene mutation status by next-generation sequencing: findings from the CAPRI-GOIM trial. Ann Oncol 2014;25:1756–61.
29. Bokemeyer C, Kohne C-H, Ciardiello F, Lenz H-J, Heinemann V, Klinkhardt U, et al. Treatment outcome according to tumor RAS mutation status in OPUS study patients with metastatic colorectal cancer (mCRC) randomized to FOLFOX4 with/without cetuximab. J Clin Oncol 2014;32:abstract 3505.
30. Lenz H, Niedzwiecki D, Innocenti F, Blanke C, Mahon MR, O'Neil BH, et al. CALGB/SWOG 80405: phase III trial of irinotecan/5-FU/leucovorin (FOLFIRI) or of oxaliplatin/5-FU/leucovorin (mFOLFOX6) with bevacizumab (BV) or cetuximab (CET) for patients (pts) with untreated metastatic adenocarcinoma of the colon or rectum (mCRC): expanded RAS analyses. Ann Oncol 2014;25:abstract 5010.
31. Price TJ, Bruhn M, Lee C, Hardingham J, Townsend AR, Mann K, et al. Correlation of PI3KCA and extended RAS gene mutation status with outcomes from the phase III AGITG MAX involving capecitabine (C) alone or in combination with bevacizumab (B) with or without mitomycin C (M) in advanced colorectal cancer (CRC). J Clin Oncol 2014;32:abstract 3539.
32. Ciardiello F, Lenz H-J, Kohne C-H, Heinemann V, Tejpar S, Melezinek I, et al. Treatment outcome according to tumor RAS mutation status in CRYSTAL study patients with metastatic colorectal cancer (mCRC) randomized to FOLFIRI with/without cetuximab. J Clin Oncol 2014;32:abstract 3506.
33. Pentheroudakis G, Kotoula V, De Roock W, Kouvatseas G, Papakostas P, Makatsoris T, et al. Biomarkers of benefit from cetuximab-based therapy in metastatic colorectal cancer: interaction of EGFR ligand expression with RAS/RAF, PIK3CA genotypes. BMC Cancer 2013;13:49.
34. NCCN clinical practice guidelines in oncology: colon cancer v.3.2014. National Comprehensive Cancer Network. [cited Feb 11, 2014]. Available from: http://www.nccn.org/professionals/physician_gls/pdf/colon.pdf.
35. European Society of Pathology. Colon External Quality Assessment Scheme. [cited Jul 18, 2014]. Available from: http://kras.eqascheme.org.
36. Van Cutsem E, Cervantes A, Nordlinger B, Arnold D. Metastatic colorectal cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol 2014;25(Suppl 3): iii1–iii9.
37. Wong NA, Gonzalez D, Salto-Tellez M, Butler R, Diaz-Cano SJ, Ilyas M, et al. RAS testing of colorectal carcinoma—a guidance document from the Association of Clinical Pathologists Molecular Pathology and Diagnostics Group. J Clin Pathol 2014;67:751–7.
38. Bendell JC, Atreya CE, André T, Tabernero J, Gordon MS, Bernards R, et al. Efficacy and tolerability in an open-label phase I/II study of MEK inhibitor trametinib (T), BRAF inhibitor dabrafenib (D), and anti-EGFR antibody

Published OnlineFirst September 4, 2015; DOI: 10.1158/1078-0432.CCR-15-0526



panitumumab (P) in combination in patients (pts) with BRAF V600E mutated colorectal cancer (CRC). J Clin Oncol 2014;32:abstract 3515.
39. Hong DS, Morris VK, Fu S, Overman MJ, Piha-Paul SA, Kee BK, et al. Phase 1B study of vemurafenib in combination with irinotecan and cetuximab in patients with BRAF-mutated advanced cancers and metastatic colorectal cancer. J Clin Oncol 2014;32:abstract 3516.
40. Sotelo MJ, Garcia-Paredes B, Aguado C, Sastre J, Diaz-Rubio E. Role of cetuximab in first-line treatment of metastatic colorectal cancer. World J Gastroenterol 2014;20:4208–19.
41. Piessevaux H, Buyse M, Schlichting M, Van Cutsem E, Bokemeyer C, Heeger S, et al. Use of early tumor shrinkage to predict long-term outcome in metastatic colorectal cancer treated with cetuximab. J Clin Oncol 2013; 31:3764–75.
42. Mansmann UR, Sartorius U, Ruediger PL, Giessen CA, Esser R, Heinemann V. Deepness of response: a quantitative analysis of its impact on post-progression survival time after first-line treatment in patients with mCRC. J Clin Oncol 2013;31:abstract 427.
43. Maughan TS, Adams RA, Smith CG, Meade AM, Seymour MT, Wilson RH, et al. Addition of cetuximab to oxaliplatin-based first-line combination chemotherapy for treatment of advanced colorectal cancer: results of the randomised phase 3 MRC COIN trial. Lancet 2011;377:2103–14.
44. Seymour MT, Brown SR, Middleton G, Maughan T, Richman S, Gwyther S, et al. Panitumumab and irinotecan versus irinotecan alone for patients with KRAS wild-type, fluorouracil-resistant advanced colorectal cancer (PICCOLO): a prospectively stratified randomised trial. Lancet Oncol 2013;14:749–59.
45. Sorich MJ, Wiese MD, Rowland A, Kichenadasse G, McKinnon RA, Karapetis CS. Extended RAS mutations and anti-EGFR monoclonal antibody survival benefit in metastatic colorectal cancer: a meta-analysis of randomized controlled trials. Ann Oncol 2014;26:13–21.

Published OnlineFirst September 4, 2015; DOI: 10.1158/1078-0432.CCR-15-0526



Clinical Cancer Research

# Analysis of *KRAS*/*NRAS* Mutations in a Phase III Study of Panitumumab with FOLFIRI Compared with FOLFIRI Alone as Second-line Treatment for Metastatic Colorectal Cancer

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*Clin Cancer Res* Published OnlineFirst September 4, 2015.