ORIGINAL ARTICLE

Panitumumab–FOLFOX4 Treatment and *RAS* Mutations in Colorectal Cancer

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ABSTRACT

BACKGROUND

Patients with metastatic colorectal cancer that harbors *KRAS* mutations in exon 2 do not benefit from anti–epidermal growth factor receptor (EGFR) therapy. Other activating *RAS* mutations may also be negative predictive biomarkers for anti-EGFR therapy.

METHODS

In this prospective–retrospective analysis, we assessed the efficacy and safety of panitumumab plus oxaliplatin, fluorouracil, and leucovorin (FOLFOX4) as compared with FOLFOX4 alone, according to *RAS* (*KRAS* or *NRAS*) or *BRAF* mutation status. A total of 639 patients who had metastatic colorectal cancer without *KRAS* mutations in exon 2 had results for at least one of the following: *KRAS* exon 3 or 4; *NRAS* exon 2, 3, or 4; or *BRAF* exon 15. The overall rate of ascertainment of *RAS* status was 90%.

RESULTS

Among 512 patients without *RAS* mutations, progression-free survival was 10.1 months with panitumumab–FOLFOX4 versus 7.9 months with FOLFOX4 alone (hazard ratio for progression or death with combination therapy, 0.72; 95% confidence interval [CI], 0.58 to 0.90; P=0.004). Overall survival was 26.0 months in the panitumumab–FOLFOX4 group versus 20.2 months in the FOLFOX4-alone group (hazard ratio for death, 0.78; 95% CI, 0.62 to 0.99; P=0.04). A total of 108 patients (17%) with nonmutated *KRAS* exon 2 had other *RAS* mutations. These mutations were associated with inferior progression-free survival and overall survival with panitumumab–FOLFOX4 treatment, which was consistent with the findings in patients with *KRAS* mutations in exon 2. *BRAF* mutations were a negative prognostic factor. No new safety signals were identified.

CONCLUSIONS

Additional *RAS* mutations predicted a lack of response in patients who received panitumumab–FOLFOX4. In patients who had metastatic colorectal cancer without *RAS* mutations, improvements in overall survival were observed with panitumumab–FOLFOX4 therapy. (Funded by Amgen and others; PRIME ClinicalTrials.gov number, NCT00364013.)

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KRAS mutation is an established predictive biomarker of resistance to anti–epidermal growth factor receptor (EGFR) therapy in patients with metastatic colorectal cancer.[1-4] Specifically, patients with *KRAS* mutations in exon 2 do not have a response to anti-EGFR therapy and may have inferior outcomes if this therapy is combined with an oxaliplatin-containing chemotherapy regimen.[2,5] More accurate selection of patients according to the genetic status of the tumor may improve the benefit–risk profile of anti-EGFR therapy.

Activating mutations in *RAS* (*KRAS* or *NRAS*) in addition to *KRAS* mutations in exon 2 have been suggested as negative predictive biomarkers for anti-EGFR therapy. This is biologically plausible on the basis of the existing biochemical and mutational data. *KRAS* and *NRAS* are closely related *RAS* oncogene family members, and mutations in either gene at codons 12, 13, 61, 117, and 146 result in increased levels of guanosine triphosphate–bound RAS proteins.[6,7] In addition, colorectal tumors harbor *KRAS* and *NRAS* mutations at these codons, and mutations tend to be mutually exclusive; this suggests functional redundancy.[8] Mutations in *HRAS*, the third member of the *RAS* family, occur infrequently in colorectal cancer.[8,9]

Clinical data have also implicated RAS genes as negative predictive biomarkers. In a randomized phase 3 study of panitumumab monotherapy[10] and other studies,[11-15] most patients with metastatic colorectal-cancer tumors harboring a mutation in *KRAS* or *NRAS* did not have a response to anti-EGFR therapy.

BRAF mutations are typically exclusive of *RAS* mutations, and the clinical data suggest that the *BRAF V600E* mutation is prognostic of patient outcome with respect to survival, but not clearly predictive of treatment effects with anti-EGFR agents, in patients with metastatic colorectal cancer.[16-19] Although no objective responses to panitumumab or cetuximab monotherapy have been reported in patients with metastatic colorectal cancer and *BRAF* mutations,[10,20] the low prevalence of such mutations makes it difficult to evaluate them as predictive biomarkers.

Previous studies of anti-EGFR therapies combined with oxaliplatin-containing regimens have shown negative outcomes in subgroups of patients with *KRAS* mutations in exon 2. Identification of other biomarker-defined subgroups with similar outcomes would influence the choice of therapy.[2,5] Here, we present the results of a prospective–retrospective biomarker analysis of the treatment effect of the full spectrum of currently characterized *RAS* (*KRAS* and *NRAS*) and *BRAF* mutations on progression-free survival and overall survival in a randomized phase 3 study of panitumumab plus oxaliplatin, fluorouracil, and leucovorin (FOLFOX4) as compared with FOLFOX4 alone in patients with previously untreated metastatic colorectal cancer.

METHODS

STUDY DESIGN AND OVERSIGHT

The Panitumumab Randomized Trial in Combination with Chemotherapy for Metastatic Colorectal Cancer to Determine Efficacy (PRIME) compared the efficacy and safety of panitumumab–FOLFOX4 with those of FOLFOX4 alone in the first-line treatment of patients, according to *KRAS* exon 2 status. The primary end point was progression-free survival. The secondary end points included overall survival and safety.[2]

The study was designed by the sponsor, Amgen, in collaboration with the first author and the study steering committee. Clinical data were collected by the investigators, and sequencing analysis was conducted by Transgenomic under the direction of the sponsor. The sponsor performed all statistical analyses. All authors vouch for the accuracy of the data and analyses and for the fidelity of this report to the protocol, which is available with the full text of this article at NEJM.org. The preliminary draft of the manuscript was written by the second author with the assistance of a medical writer who was paid by the sponsor. Subsequent drafts were revised and reviewed by all the authors. All the authors made the decision to submit the manuscript for publication.

TUMOR SPECIMENS

Banked tumor specimens that were characterized as nonmutated *KRAS* exon 2 on the basis of an assay for investigational use only (TheraScreen *KRAS* Mutation Kit, Qiagen; LightCycler, Roche) were selected for analysis.[2] DNA was extracted from formalin-fixed, paraffin-embedded tumor specimens with the use of a DNA Extraction Mini Kit (Qiagen). Specimens that contained less than 50% tumor area were macrodissected. In a few cases, DNA was extracted from stored

slides that had been stained for immunohistochemical analysis.

MUTATIONAL ANALYSIS

Mutations in *KRAS* exon 3 (at codon 61) and exon 4 (at codons 117 and 146); *NRAS* exon 2 (at codons 12 and 13), exon 3 (at codon 61), and exon 4 (at codons 117 and 146); and *BRAF* exon 15 (at codon 600) were prespecified on the basis of previous studies.[7,14,21,22] Gene alterations that were not prespecified (e.g., *KRAS* and *NRAS* exon 3 [codon 59] mutations) were analyzed as exploratory end points. Polymerase-chain-reaction (PCR) primer sequences amplified regions up to 200 bp in length to account for the fragmented nature of DNA in formalin-fixed, paraffin-embedded specimens. Separate data sets were generated by means of bidirectional Sanger sequencing and WAVE-based Surveyor Scan Kits (Transgenomic).[23-26] Double-stranded PCR amplicons were melted and cooled to form a heteroduplex–homoduplex mixture that was treated with Surveyor nuclease. The resulting DNA fragments were analyzed with the use of high-performance liquid chromatography (WAVE HS System). The formation of mutant:nonmutant heteroduplexes resulted in fragments of various sizes. The testing plan and methods were prespecified, and investigators in the testing laboratory were unaware of treatment assignments and clinical outcomes. Bidirectional Sanger sequencing and testing with WAVE-based Surveyor Scan Kits were validated according to the Clinical Laboratory Improvement Amendments of 1988.

STATISTICAL ANALYSIS

The statistical analysis plan was prespecified before the *RAS* and *BRAF* testing results became available. Two clinical data snapshots were used: the primary analysis (prespecified to be performed when >50% of patients with nonmutated *KRAS* exon 2 had died from any cause) and the updated analysis of overall survival (an exploratory analysis that was undertaken when >80% of patients in both the nonmutated and mutated *KRAS* exon 2 subgroups had died from any cause), which provided the most up-to-date estimate of overall survival in the PRIME study.

The primary objective of the current prospective–retrospective analysis was to evaluate the treatment effect of panitumumab—FOLFOX4 as compared with FOLFOX4 alone in patients without *RAS* mutations (nonmutated *KRAS* and *NRAS* exons 2, 3, and 4) and in those without *RAS* and *BRAF* mutations (nonmutated *KRAS* and *NRAS* exons 2, 3, and 4, and *BRAF* exon 15) in the primary-analysis population of the PRIME study. Subsequent evaluation of the treatment effect on the basis of the updated overall-survival data was similarly prespecified in the statistical analysis plan, but only the results of the overall-survival end point are reported, since data collection was limited to survival information. Patients were characterized as having *RAS* mutations if any predefined activating mutation in *KRAS* or *NRAS* was detected, and patients were characterized as having *RAS* or *BRAF* mutations if any predefined *RAS* or *BRAF* mutation was detected.

The hypothesis testing in this analysis was exploratory in nature. An overall 5% significance level was used to compare the treatment effect on progression-free survival and overall survival in subpopulations without *RAS* mutations and in subpopulations without *RAS* and *BRAF* mutations. To control the overall type 1 error rate, a sequential testing scheme was used for evaluation of the treatment effects of panitumumab on progression-free survival among patients with nonmutated *RAS* and nonmutated *RAS* and *BRAF,* followed by a test of the treatment effects on overall survival among patients in the same subgroups. No hypothesis testing was conducted in the subgroups with mutations. To estimate the treatment effects of panitumumab, we used Cox proportional-hazards models stratified according to randomization factors, with all randomly assigned patients in each biomarker subgroup included in the assessment. A log-rank test stratified according to randomization factors was used to compare the treatment effects on progression-free survival and overall survival in the panitumumab–FOLFOX4 group with the treatment effects on progression-free survival and overall survival in the FOLFOX4-alone group. Sensitivity analyses, including a multivariate Cox model and propensity-score analysis, were used to confirm the primary results. Interaction tests were performed to compare the treatment effects of panitumumab between the subgroup with nonmutated *RAS* and the subgroup with mutated *RAS* and between the subgroup with nonmutated *RAS* and the subgroup with nonmutated *KRAS* in exon 2 and other *RAS* mutations. Multivariate Cox models were also used to explore the prognostic relevance of baseline covariates.

RESULTS

PATIENTS

Of the 1183 patients who underwent randomization, 1096 (93%) had previously been evaluated for *KRAS* exon 2 (656 patients without *KRAS* mutations in exon 2 [60%] and 440 patients with *KRAS* mutations in exon 2 [40%]) (Fig. S1 in the Supplementary Appendix, available at NEJM.org).[2] The status of *KRAS* exon 3 or 4; *NRAS* exon 2, 3, or 4; or *BRAF* exon 15 (Table 1, and Table S1 in the Supplementary Appendix) was determined in 639 of the 656 patients without *KRAS* mutations in exon 2. Identical results were obtained by means of bidirectional Sanger sequencing and WAVE-based Surveyor analysis.

RAS status was ascertained in 1060 of the 1183 patients (90%) who underwent randomization. Of these 1060 patients, 512 (48%) were identified as having tumors with nonmutated *RAS* (no *KRAS* or *NRAS* mutations in exons 2, 3, or 4) and 548 (52%) were identified as having tumors with mutated *RAS* (any *KRAS* or *NRAS* mutations in exon 2, 3, or 4) (Fig. S1 in the Supplementary Appendix). Of 620 patients with data that could be evaluated for *RAS*, 108 (17%) who were originally categorized as not having *KRAS* mutations in exon 2 had other *RAS* mutations. Baseline clinical and demographic characteristics, including race or ethnic group, age, Eastern Cooperative Oncology Group (ECOG) performance-status score (on a scale from 0 to 5, with 0 indicating no symptoms and full activity and higher scores indicating increasing levels of disability),[27] primary tumor type, and number of metastatic lesions were generally similar between patients with nonmutated *RAS* and those with mutated *RAS* and were consistent with the reported results for *KRAS* exon 2 in patients with metastatic colorectal-cancer tumors.[2]

The rate of ascertainment of *RAS* and *BRAF* status was 89% (assessed in 1047 of 1183 patients). Of 619 patients without *KRAS* mutations in exon 2 who could be evaluated for *BRAF*, 53 (9%) had *V600E* mutations. Mutations in *BRAF* exon 15 were mutually exclusive of *KRAS* and *NRAS* mutations in patients without *KRAS* mutations in exon 2 who could be evaluated. The proportion of patients with each of the *RAS* or *BRAF* mutations (Table 1) was consistent with that reported in a recently published article.[21]

EFFICACY ACCORDING TO TUMOR *RAS* STATUS

At the time of the primary analysis (data-cutoff point, August 29, 2009), 54% of the patients had died.[2] In patients without *KRAS* mutations in exon 2 who received panitumumab–FOLFOX4, as compared with those who received FOLFOX4 alone, there was a significant improvement in progression-free survival (9.6 vs. 8.0 months, P=0.02) and a 4.2-month improvement in overall survival, which was not significant (23.9 vs. 19.7 months, P=0.07) (Table 2). In an exploratory, updated analysis of overall survival (data-cutoff point, January 24, 2013), 82% of the patients had died. On the basis of this analysis, panitumumab–FOLFOX4 was associated with a 4.4-month improvement in overall survival (23.8 months in the panitumumab–FOLFOX4 group vs. 19.4 months in the FOLFOX4-alone group, P=0.03).

These analyses were extended to evaluate the predictive value of mutations other than *KRAS* mutations in exon 2. In the subgroup of patients without *RAS* mutations (the primary-analysis population), panitumumab—FOLFOX4, as compared with FOLFOX4 alone, was associated with a significant improvement in progression-free survival (10.1 vs. 7.9 months, P=0.004) and a significant 5.8-month improvement in overall survival (26.0 vs. 20.2 months, P=0.04) (Table 2 and Fig. 1 and 2A and 2B). Consistently, significant results were observed in the exploratory, updated overall-survival analysis with respect to the magnitude of improvement with panitumumab–FOLFOX4 as compared with FOLFOX4 alone (Table 2 and Fig. 2C).

A total of 17% of patients without *KRAS* mutations in exon 2 had mutations in other *RAS* exons. In this subgroup of 108 patients, outcomes in the primary analysis and in the exploratory updated analysis of overall survival showed that progression-free survival (Fig. S2A in the Supplementary Appendix) and overall survival (Fig. S2B and S2C in the Supplementary Appendix) were shorter in the panitumumab–FOLFOX4 group than in the FOLFOX4-alone group, though the difference was not significant. These outcomes were consistent with those observed in the subgroup of patients with *KRAS* mutations in exon 2; in this subgroup, progression-free survival in the primary analysis was significantly shorter in the panitumumab–FOLFOX4 group than in the FOLFOX4-alone group (7.3 months

Table 1. *RAS* and *BRAF* Mutation Status.*

Variable	Panitumumab–FOLFOX4	FOLFOX4 Alone	Total
KRAS exon 2 at codons 12 and 13 — no. of patients			
Nonmutated	325	331	656
Mutated†	221	219	440
KRAS exon 2 tumors tested for *RAS* and *BRAF* — no./total no. (%)‡			
KRAS exon 3 at codon 61			
Nonmutated	308/320 (96)	306/321 (95)	614/641 (96)
Mutated	10/320 (3)	14/321 (4)	24/641 (4)
Not determined	2/320 (1)	1/321 (<1)	3/641 (0)
KRAS exon 4 at codon 117 or 146			
Nonmutated	288/320 (90)	296/321 (92)	584/641 (91)
Mutated	21/320 (7)	15/321 (5)	36/641 (6)
Not determined	11/320 (3)	10/321 (3)	21/641 (3)
NRAS exon 2 at codon 12 or 13			
Nonmutated	308/320 (96)	307/321 (96)	615/641 (96)
Mutated	8/320 (2)	14/321 (4)	22/641 (3)
Not determined	4/320 (1)	0/321 (0)	4/641 (1)
NRAS exon 3 at codon 61			
Nonmutated	305/320 (95)	305/321 (95)	610/641 (95)
Mutated	12/320 (4)	14/321 (4)	26/641 (4)
Not determined	3/320 (1)	2/321 (1)	5/641 (1)
NRAS exon 4 at codon 117 or 146			
Nonmutated	316/320 (99)	313/321 (98)	629/641 (98)
Mutated	0/320 (0)	0/321 (0)	0/641 (0)
Not determined	4/320 (1)	8/321 (2)	12/641 (2)
BRAF exon 15 at codon 600			
Nonmutated	286/320 (89)	280/321 (87)	566/641 (88)
Mutated	24/320 (8)	29/321 (9)	53/641 (8)
Not determined	10/320 (3)	12/321 (4)	22/641 (3)
All patients who underwent randomization — no.	593	590	1183
Ascertainment of mutation status — no./total no. (%)			
RAS	531/593 (90)	529/590 (90)	1060/1183 (90)§
BRAF	310/593 (52)	309/590 (52)	619/1183 (52)
RAS and *BRAF*	524/593 (88)	523/590 (89)	1047/1183 (89)¶

* FOLFOX4 denotes oxaliplatin, fluorouracil, and leucovorin.
† A total of 440 patients with *KRAS* mutations in exon 2 were not retested for *RAS* or *BRAF*.
‡ Of 641 samples tested, 2 did not yield a result (and did not have a mutation) in at least one *RAS* or *BRAF* exon. Samples that had any *RAS* exon mutation, regardless of whether other *RAS* exons did not yield a result, were characterized as mutant *RAS* and thus could be evaluated for *RAS* mutation status.
§ The total includes 440 patients with *KRAS* mutations in exon 2 and 620 patients with data that could be evaluated for *RAS*. Of 641 samples tested, 21 did not yield a result (and did not have a mutation) in at least one *RAS* exon.
¶ Of 1060 samples with data that could be evaluated for *RAS*, an additional 13 did not yield a *BRAF* result.

Table 2. Efficacy Results According to *RAS* Mutation Status.

Variable	Panitumumab–FOLFOX4	FOLFOX4 Alone	Hazard Ratio (95% CI)	P Value	P Value for Interaction Test*
No *KRAS* mutation in exon 2					
No. of patients	325	331			
Months of progression-free survival in primary analysis — median (95% CI)	9.6 (9.2–11.1)	8.0 (7.5–9.3)	0.80 (0.66–0.97)	0.02	
Months of overall survival — median (95% CI)					
Primary analysis	23.9 (20.3–28.3)	19.7 (17.6–22.6)	0.83 (0.67–1.02)	0.07	
Updated analysis	23.8 (20.0–27.7)	19.4 (17.4–22.6)	0.83 (0.70–0.98)	0.03	
***KRAS* mutation in exon 2**					
No. of patients	221	219			
Months of progression-free survival in primary analysis — median (95% CI)	7.3 (6.3–8.0)	8.8 (7.7–9.4)	1.29 (1.04–1.62)	0.02	
Months of overall survival — median (95% CI)					
Primary analysis	15.5 (13.1–17.6)	19.3 (16.5–21.8)	1.24 (0.98–1.57)	0.07	
Updated analysis	15.5 (13.1–17.6)	19.2 (16.2–21.5)	1.16 (0.94–1.41)	0.16	
No *RAS* mutation					
No. of patients	259	253			
Months of progression-free survival in primary analysis — median (95% CI)	10.1 (9.3–12.0)	7.9 (7.2–9.3)	0.72 (0.58–0.90)	0.004	
Months of overall survival — median (95% CI)					
Primary analysis	26.0 (21.7–30.4)	20.2 (17.7–23.1)	0.78 (0.62–0.99)	0.04	
Updated analysis	25.8 (21.7–29.7)	20.2 (17.6–23.6)	0.77 (0.64–0.94)	0.009	
No *KRAS* mutation in exon 2, other *RAS* mutation					
No. of patients	51	57			
Months of progression-free survival in primary analysis — median (95% CI)	7.3 (5.3–9.2)	8.0 (6.4–11.3)	1.28 (0.79–2.07)	0.33	0.04
Months of overall survival — median (95% CI)					
Primary analysis	17.1 (10.8–19.4)	18.3 (13.0–23.2)	1.29 (0.79–2.10)	0.31	0.07
Updated analysis	17.1 (10.8–19.4)	17.8 (13.0–23.2)	1.39 (0.91–2.13)	0.12	0.01
***RAS* mutation**					
No. of patients	272	276			
Months of progression-free survival in primary analysis — median (95% CI)	7.3 (6.3–7.9)	8.7 (7.6–9.4)	1.31 (1.07–1.60)	0.008	<0.001
Months of overall survival — median (95% CI)					
Primary analysis	15.6 (13.4–17.9)	19.2 (16.7–21.8)	1.25 (1.02–1.55)	0.03	0.004
Updated analysis	15.5 (13.4–17.9)	18.7 (16.5–21.5)	1.21 (1.01–1.45)	0.04	0.001

* The interaction test is for the comparison with nonmutated *RAS*.

vs. 8.8 months, P=0.02) (Table 2). In the primary analysis, interaction testing between the subgroups that did not have *RAS* mutations and the subgroups that did not have *KRAS* mutations in exon 2 but did have other *RAS* mutations was significant for progression-free survival (P=0.04) but not for overall survival (P=0.07). In the updated analysis of overall survival, which was based on a larger number of deaths from any cause, the results of interaction testing were significant (P=0.01). These results indicate that treatment effects differed between the subgroups of patients without *RAS* mutations and those without *KRAS* mutations in exon 2 but with other

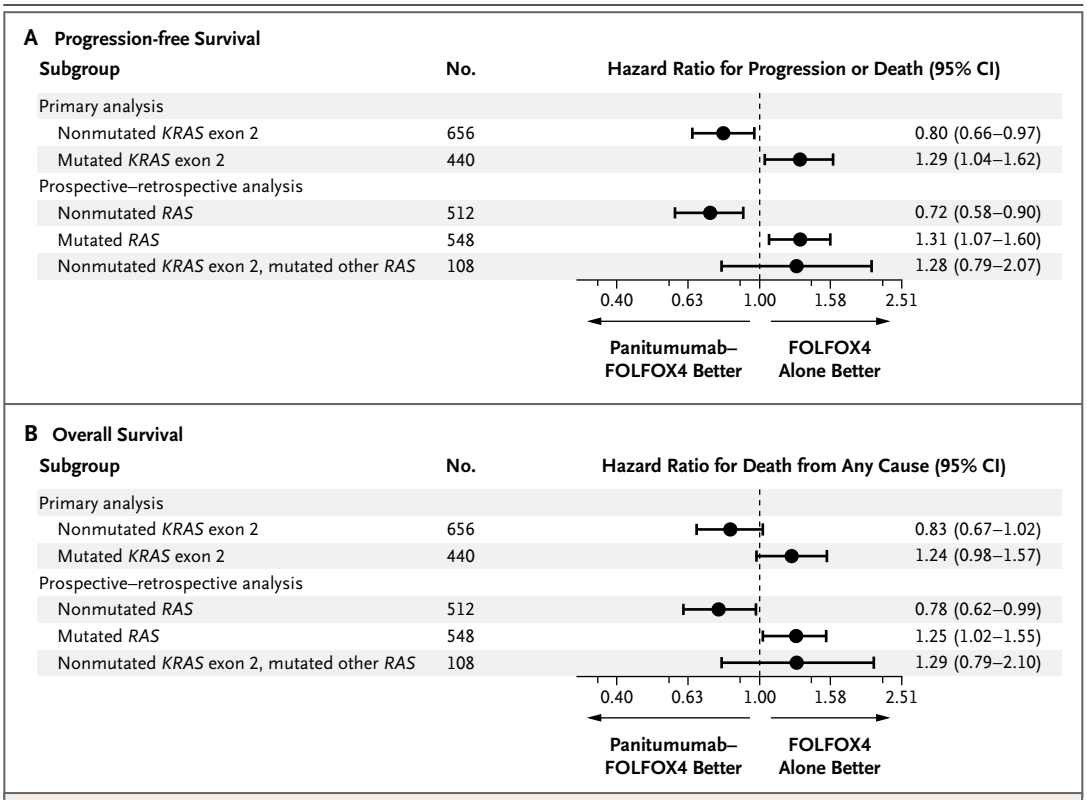


Figure 1. Hazard Ratio for Disease Progression or Death and Hazard Ratio for Death from Any Cause, According to *KRAS* and *RAS* Mutation Status.

RAS mutations, suggesting that *RAS* mutations, in addition to *KRAS* mutations in exon 2, were negative predictive factors (Table 2).

In the expanded subgroup of patients with mutated *RAS* tumors, progression-free survival (Fig. S3A in the Supplementary Appendix) and overall survival (Fig. S3B and S3C in the Supplementary Appendix) were significantly shorter in the panitumumab–FOLFOX4 group than in the FOLFOX4-alone group in the primary analysis and in the exploratory, updated analysis of overall survival. Interaction testing for progression-free survival and overall survival was significant in all data sets, further suggesting that *RAS* mutations had a negative predictive value (Table 2). Additional efficacy results for patients with data that could not be evaluated for *RAS* are shown in Table S2 in the Supplementary Appendix.

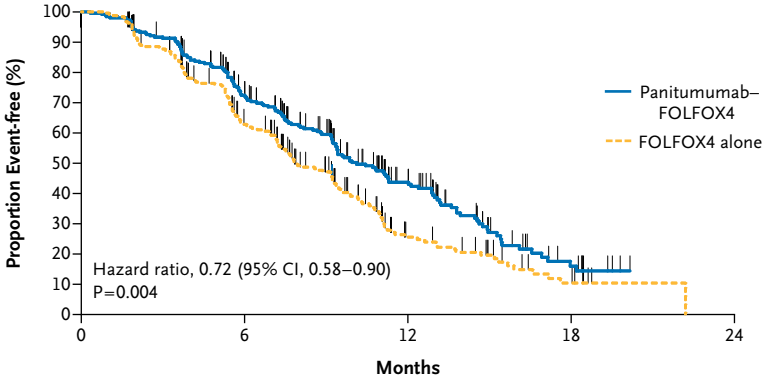
The treatment effect on progression-free survival and overall survival in favor of panitumumab–FOLFOX4 in patients with nonmutated *RAS* was observed across subpopulations predefined according to baseline covariates, except an ECOG performance-status score of 2 (Fig. S4 in the Supplementary Appendix).

Subsequent to the prespecified analysis, previously reported mutations in *KRAS* and *NRAS* at codon 59 (A59G and A59T)[9,28-30] were identified in seven patients. In an exploratory analysis involving this small patient population, exclusion of these mutated alleles slightly improved progression-free survival and overall survival (Table S3 and Fig. S5 in the Supplementary Appendix).

EFFICACY ACCORDING TO TUMOR *BRAF* STATUS

In the nonmutated *RAS* and nonmutated *BRAF* subgroup, panitumumab–FOLFOX4 was associated with a 1.6-month improvement in progression-free survival and a 7.4-month improvement in overall survival, as compared with FOLFOX4 alone (Table 3). The minor differences between FOLFOX4–panitumumab and FOLFOX4 alone in the subgroup of patients without *RAS* mutations but with *BRAF* mutations were not significant (Fig. S6 in the Supplementary Appendix).

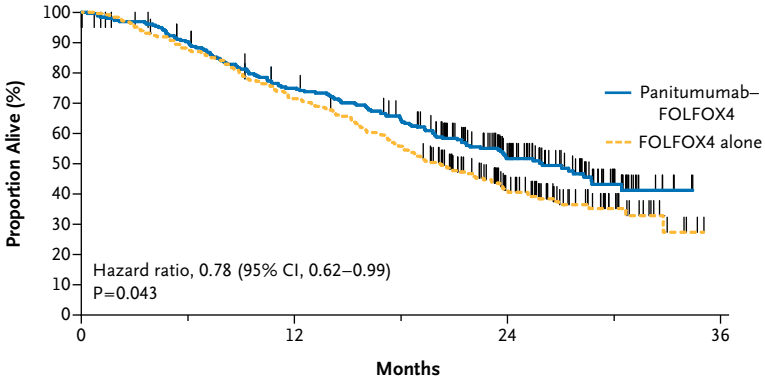
A Progression-free Survival in the Primary-Analysis Population



	Events no./total no. (%)	Median Mo (95% CI)
Panitumumab–FOLFOX4	156/259 (60)	10.1 (9.3–12.0)
FOLFOX4 alone	170/253 (67)	7.9 (7.2–9.3)

No. at Risk				
Panitumumab–FOLFOX4	259	171	65	10
FOLFOX4 alone	253	140	31	7

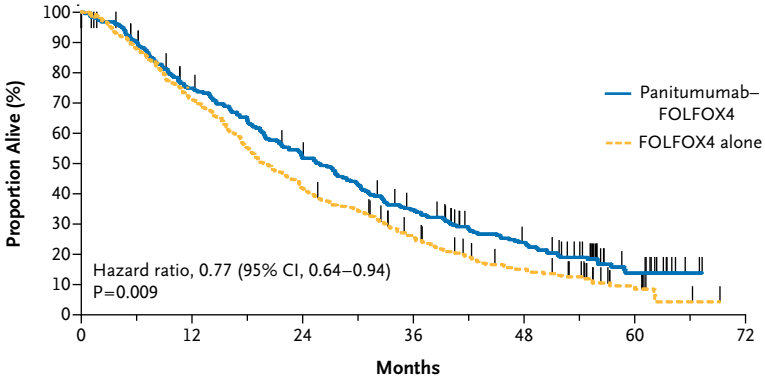
B Overall Survival in the Primary-Analysis Population



	Events no./total no. (%)	Median Mo (95% CI)
Panitumumab–FOLFOX4	128/259 (49)	26.0 (21.7–30.4)
FOLFOX4 alone	148/253 (58)	20.2 (17.7–23.1)

No. at Risk				
Panitumumab–FOLFOX4	259	189	88	0
FOLFOX4 alone	253	174	65	0

C Overall Survival in the Updated-Analysis Population



	Events no./total no. (%)	Median Mo (95% CI)
Panitumumab–FOLFOX4	204/259 (79)	25.8 (21.7–29.7)
FOLFOX4 alone	218/253 (86)	20.2 (17.6–23.6)

No. at Risk						
Panitumumab–FOLFOX4	259	189	129	83	49	14
FOLFOX4 alone	253	176	104	60	30	8

Figure 2. Kaplan–Meier Estimates of Progression-free Survival in the Primary-Analysis Population and Overall Survival in the Primary-Analysis and Updated-Analysis Populations, According to Treatment Group.

Table 3. Efficacy Results According to *RAS* and *BRAF* Mutation Status in the Primary-Analysis Population.*

Variable	Panitumumab–FOLFOX4	FOLFOX4 Alone	Hazard Ratio (95% CI)	P Value
No *RAS* or *BRAF* mutations				
No. of patients	228	218		
Months of progression-free survival — median (95% CI)	10.8 (9.4–12.4)	9.2 (7.4–9.6)	0.68 (0.54–0.87)	0.002
Months of overall survival — median (95% CI)	28.3 (23.7–NE)	20.9 (18.4–23.8)	0.74 (0.57–0.96)	0.02
No *RAS* mutation, *BRAF* mutation				
No. of patients	24	29		
Months of progression-free survival — median (95% CI)	6.1 (3.7–10.7)	5.4 (3.3–6.2)	0.58 (0.29–1.15)	0.12
Months of overall survival — median (95% CI)	10.5 (6.4–18.9)	9.2 (8.0–15.7)	0.90 (0.46–1.76)	0.76
***RAS* or *BRAF* mutation**				
No. of patients	296	305		
Months of progression-free survival — median (95% CI)	7.3 (6.3–7.7)	8.0 (7.5–9.0)	1.24 (1.02–1.49)	0.03
Months of overall survival — median (95% CI)	15.3 (12.7–17.6)	18.0 (15.9–20.8)	1.21 (0.99–1.47)	0.06
No *KRAS* mutation in exon 2, other *RAS* or *BRAF* mutation				
No. of patients	75	86		
Months of progression-free survival — median (95% CI)	6.7 (5.3–8.2)	7.3 (5.7–8.0)	1.05 (0.73–1.52)	0.80
Months of overall survival — median (95% CI)	14.5 (10.4–18.5)	15.8 (11.9–18.8)	1.14 (0.78–1.66)	0.51

* NE denotes not evaluated.

PROGNOSTIC EFFECTS OF *RAS* AND *BRAF* MUTATION STATUS

The prognostic effects of *RAS* and *BRAF* mutation status were evaluated by comparing the hazard ratios for death from any cause with no mutation versus mutation within each treatment group and across groups (Fig. S7 in the Supplementary Appendix). Most hazard ratios favored nonmutated status in the panitumumab–FOLFOX4 group and were neutral in the FOLFOX4-alone group. *BRAF* mutations were associated with reduced overall survival among patients without *KRAS* mutations in exon 2 and among those with *NRAS* mutations in exon 3.

SAFETY

The incidence rates, types, and severity of adverse events among patients with nonmutated *RAS* in the panitumumab–FOLFOX4 group (Table 4) were similar to those previously reported in the group of patients with nonmutated *KRAS* exon 2 who were treated with panitumumab–FOLFOX4.[2] Treatment exposure, disease-control rates, and the proportion of patients who discontinued any study drug due to an adverse event were also similar to those previously reported.[2] The safety profile for patients with *RAS* mutations was similar to that reported for patients with *KRAS* mutations in exon 2. No new safety signals were identified.

DISCUSSION

Testing for *KRAS* exon 2 tumor mutations is currently recommended to help guide decisions regarding eligibility for anti-EGFR therapy in patients with metastatic colorectal cancer. Although *KRAS* testing has facilitated the selection of patients who are most likely to have a response to anti-EGFR therapy, a substantial fraction of patients do not benefit from treatment. It is hoped

Table 4. Summary of Adverse Events, According to *RAS* Mutation Status in the Primary-Analysis Population.

Adverse Event	Nonmutated *RAS*			Mutated *RAS*		
	Panitumumab–FOLFOX4 (N=256)	FOLFOX4 Alone (N=250)	Total (N=506)	Panitumumab–FOLFOX4 (N=268)	FOLFOX4 Alone (N=275)	Total (N=543)
	number of patients (percent)					
Any adverse event	256 (100)	248 (99)	504 (100)	266 (99)	273 (99)	539 (99)
Worst grade of 3	146 (57)	124 (50)	270 (53)	153 (57)	146 (53)	299 (55)
Worst grade of 4	71 (28)	51 (20)	122 (24)	63 (24)	55 (20)	118 (22)
Worst grade of 5	14 (5)	16 (6)	30 (6)	19 (7)	10 (4)	29 (5)
Any serious adverse event	110 (43)	92 (37)	202 (40)	121 (45)	84 (31)	205 (38)
Adverse event leading to permanent discontinuation of any study drug	65 (25)	40 (16)	105 (21)	60 (22)	37 (13)	97 (18)
Not serious	48 (19)	28 (11)	76 (15)	50 (19)	24 (9)	74 (14)
Serious	24 (9)	15 (6)	39 (8)	17 (6)	14 (5)	31 (6)

that further refinement of tumor-specific genetic markers will allow more accurate selection of patients who are likely to have a response to a particular treatment and prevent toxic effects in those who are unlikely to benefit.

Biomarker exploration has been broadened to include EGFR pathway mutations, in addition to those in *KRAS* exon 2. In a retrospective biomarker analysis of a randomized phase 3 study of panitumumab monotherapy, EGFR signaling–pathway genes were assessed for their predictive ability.[10] From this study, a hypothesis was generated that activating mutations in *KRAS* or *NRAS* would be predictive of nonresponse to panitumumab therapy. The current analysis, which was based on biologic plausibility and exploratory biomarker data, further assesses the hypothesis that additional activating *RAS* mutations predict unresponsiveness to panitumumab treatment.[7,10,21,28]

Negative treatment effects of panitumumab–FOLFOX4 on progression-free survival and overall survival were observed among patients with tumors that did not have *KRAS* mutations in exon 2 but that did have other *RAS* mutations. In an interaction test, treatment effects were significantly worse than those in the group of patients with nonmutated *RAS*; this suggests that mutations in *RAS*, in addition to *KRAS* mutations in exon 2, are predictive of adverse outcomes for panitumumab–FOLFOX4 treatment. The magnitude of the treatment effect in the patients with mutated *RAS* was similar to that previously observed in patients with mutated *KRAS* exon 2[2] and further indicated that patients with tumors that harbored any activating *RAS* mutations did not benefit from and may have been harmed by panitumumab–FOLFOX4 treatment.

Among patients in the primary-analysis population who did not have *RAS* mutations, an increase in overall survival of 5.8 months was noted with the addition of panitumumab to FOLFOX4 as compared with FOLFOX4 alone. The results of the exploratory, updated analysis of overall survival were consistent with these findings. The observed incidence, types, and severity of adverse events associated with panitumumab–FOLFOX4 in the nonmutated *RAS* and mutated *RAS* subgroups were similar to the previously reported safety findings for *KRAS* in PRIME,[2] and no new safety signals were identified.

In the subgroup of patients without *RAS* and *BRAF* mutations, a 7.4-month increase in overall survival was observed in the panitumumab–FOLFOX4 group. As suggested previously,[17] *BRAF V600E* mutations appeared to confer a poor prognosis, regardless of the treatment group.

This analysis was retrospective and exploratory in nature and therefore subject to limitations. The alpha error for hypothesis testing was previously allocated to the primary analysis,[2] and *RAS* and *BRAF* status may not be representative of the intention-to-treat population from the original

randomization. However, methodologic aspects of the analysis provided a rigorous framework for evaluating *RAS* and *BRAF* as biomarkers. Tissue samples were collected with appropriate informed consent before randomization. The biomarker hypothesis was restricted to *RAS* and *BRAF*, and the statistical analysis plan was finalized before *RAS* and *BRAF* status became available. In addition, the analysis was conducted with data from a large, randomized, controlled trial.[2] The high rate of ascertainment of *RAS* status (90%) minimized the potential for ascertainment bias. Two laboratory-developed tests, validated according to the Clinical Laboratory Improvement Amendments of 1988, provided mutual confirmation.

Two interaction tests showed a clear separation of panitumumab treatment effects between nonmutated *RAS* and mutated *RAS* as well as between nonmutated *RAS* and nonmutated *KRAS* exon 2 with other *RAS* mutations; the latter finding indicates the predictive value of *RAS* mutations other than *KRAS* mutations in exon 2. These results were observed across all meaningful end points and in all relevant subgroups.

In conclusion, *RAS* mutations, in addition to *KRAS* exon 2 mutations, predict a lack of response to anti-EGFR therapy in patients with metastatic colorectal cancer. Panitumumab plus oxaliplatin-containing regimens have no value in patients with metastatic colorectal cancer and mutated *RAS*. The benefit–risk profile of panitumumab–FOLFOX4 was improved by excluding patients with mutated *RAS* metastatic colorectal-cancer tumors. Pooled trials or meta-analyses of anti-EGFR therapy are needed to confirm these findings.

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Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

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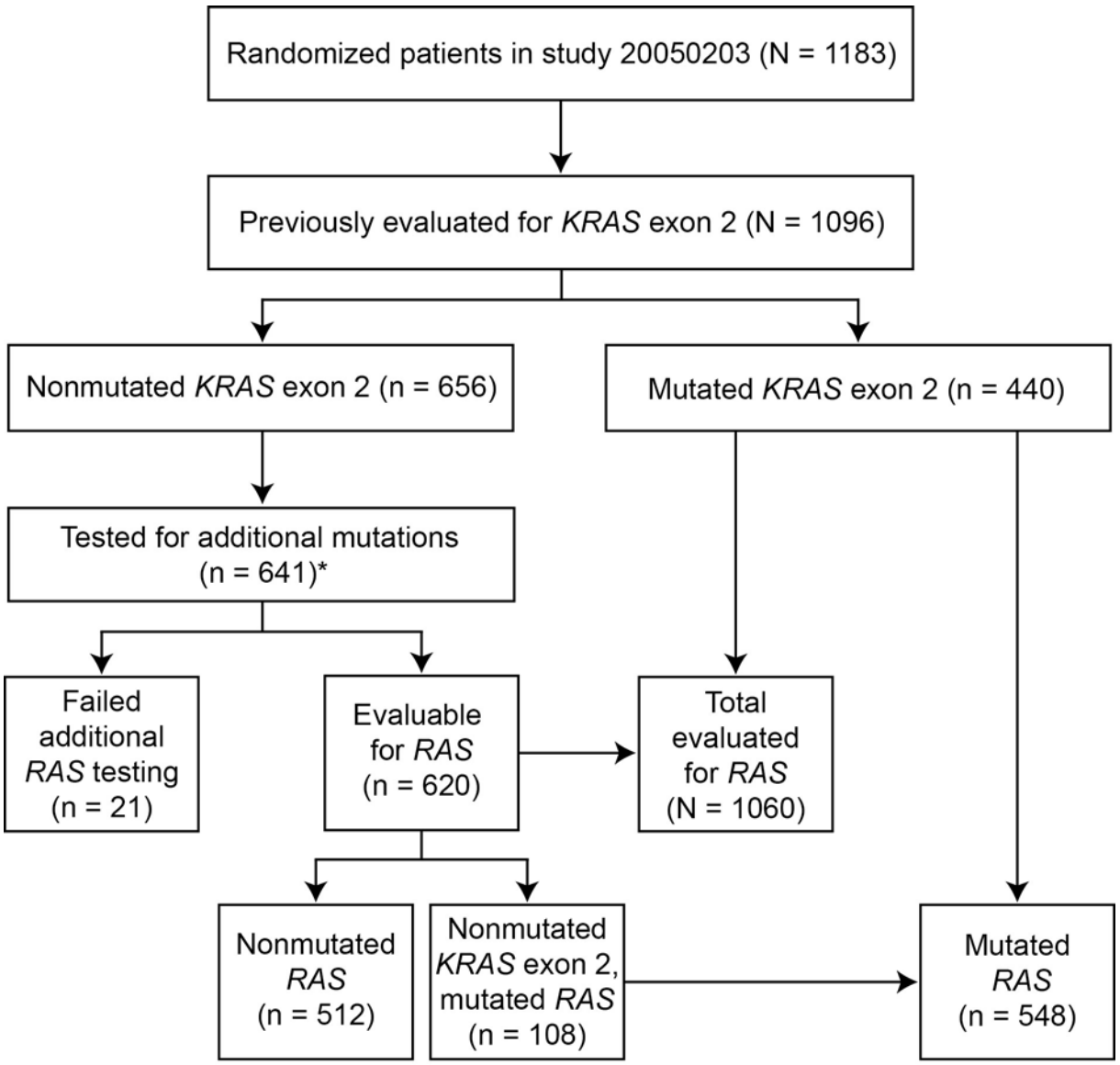
Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Douillard J-Y, Oliner KS, Siena S, et al. Panitumumab–FOLFOX4 treatment and *RAS* mutations in colorectal cancer. N Engl J Med 2013;369:1023-34. DOI: 10.1056/NEJMoa1305275

SUPPLEMENTAL MATERIALS

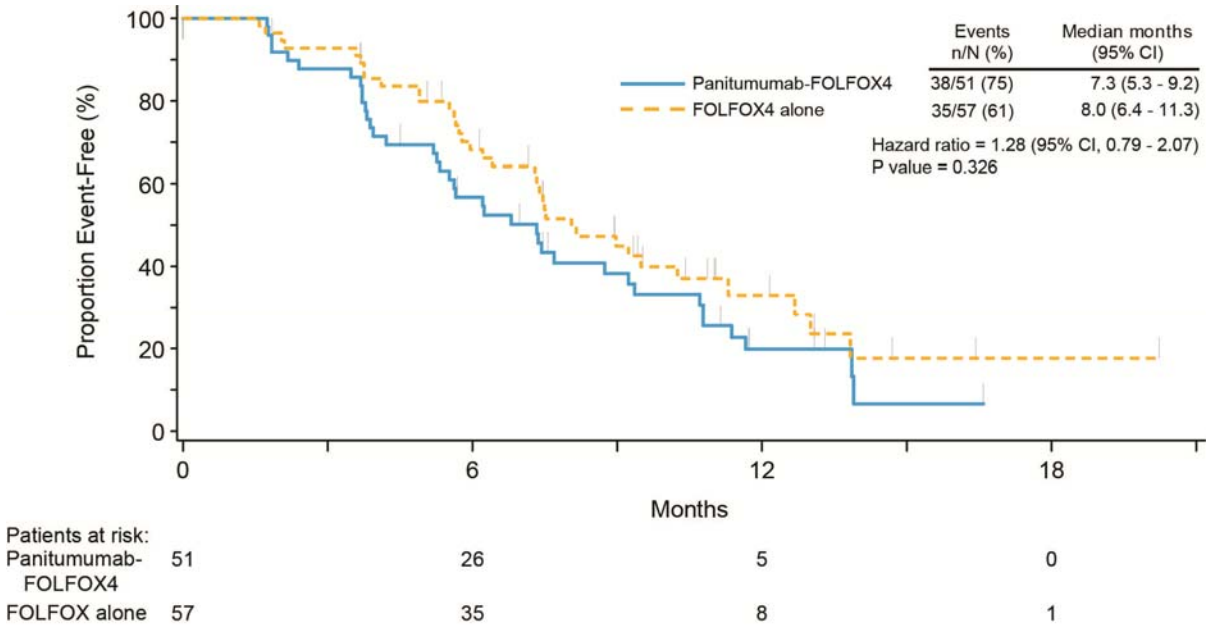
Figure S1. *RAS* Testing in Study 20050203.



*Two patient samples did not yield a result for any *RAS* or *BRAF* exon tested

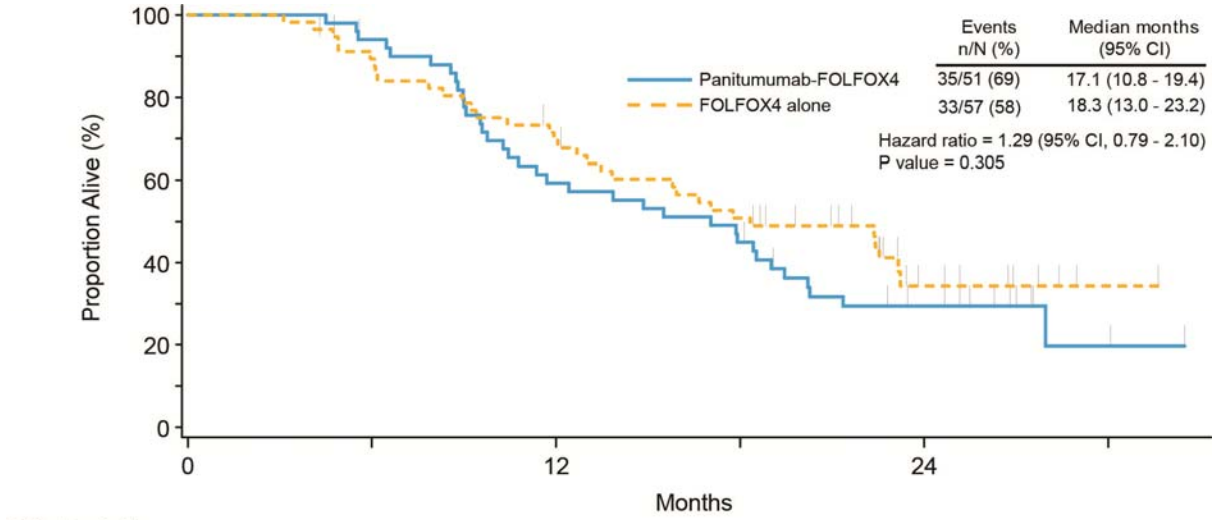
Figure S2. Kaplan-Meier Plots in Patients With Nonmutated *KRAS* Exon 2, Mutated Other *RAS* Exons Tumors. The analysis was conducted for progression-free survival on the primary analysis dataset (Panel A) and for overall survival on the primary analysis dataset (Panel B) and the updated overall survival dataset (Panel C).

A. Progression-free Survival



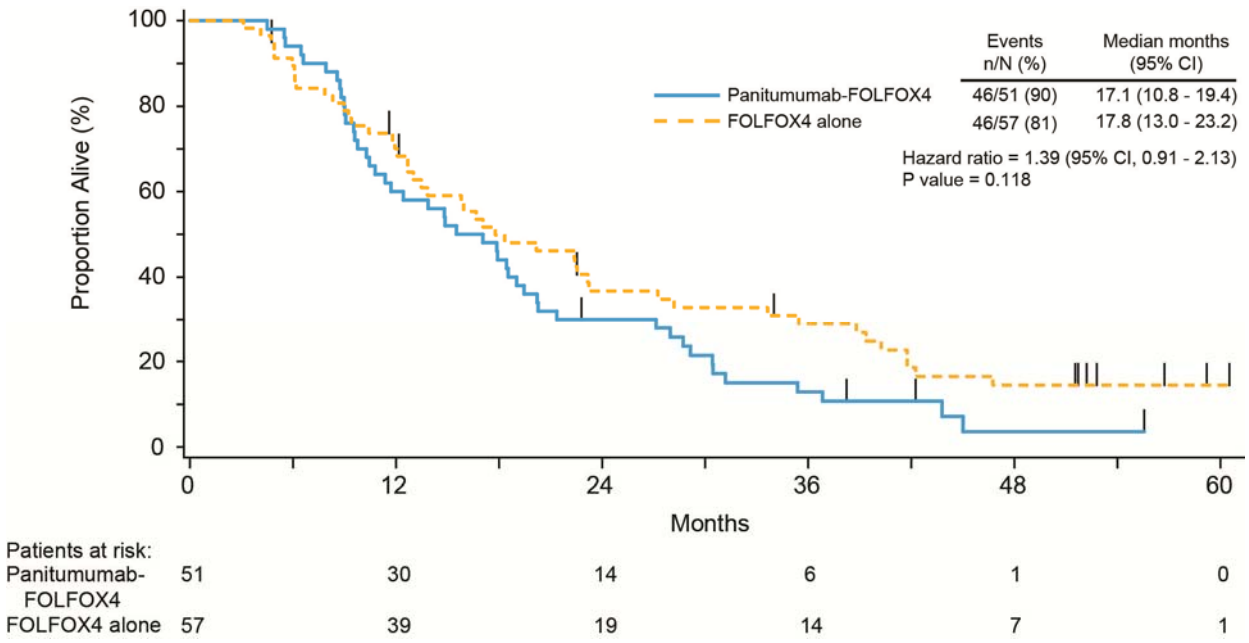
Patients at risk:				
Panitumumab-FOLFOX4	51	26	5	0
FOLFOX alone	57	35	8	1

B. Overall Survival (Primary Analysis Dataset)



Patients at risk:			
Panitumumab-FOLFOX4	51	29	11
FOLFOX4 alone	57	38	8

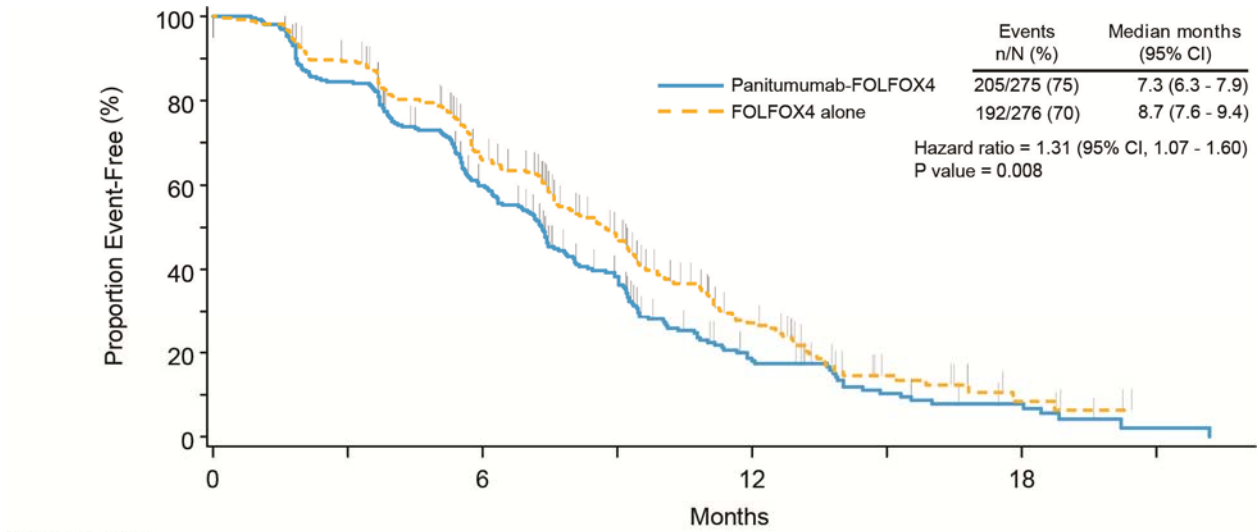
C. Overall Survival (Updated Overall Survival Analysis Dataset)



Patients at risk:						
Panitumumab-FOLFOX4	51	30	14	6	1	0
FOLFOX4 alone	57	39	19	14	7	1

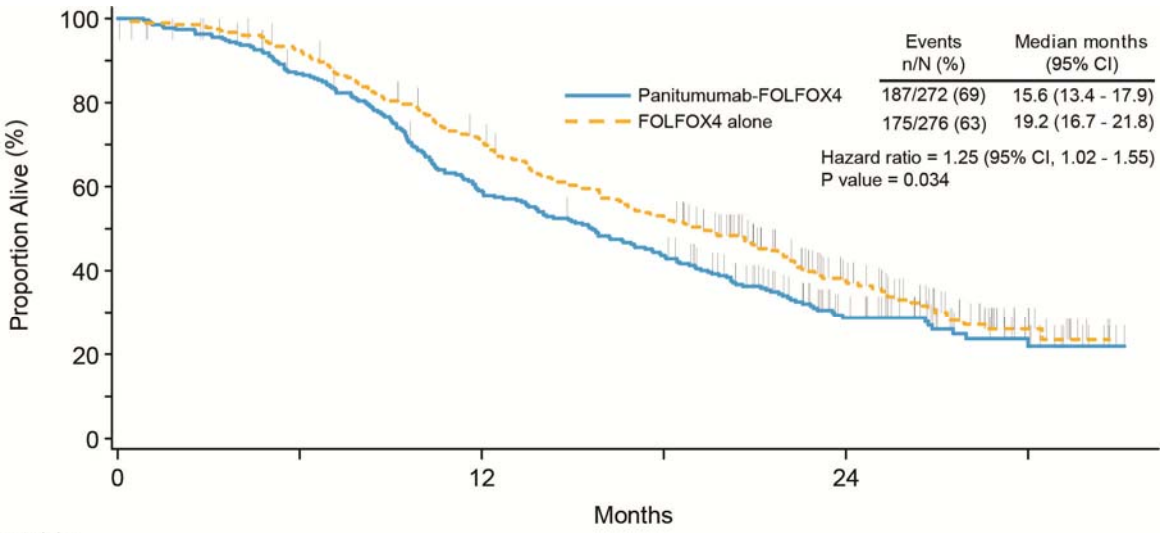
Figure S3. Kaplan-Meier Plots in Patients With Mutated *RAS* Tumors. The analysis was conducted for progression-free survival on the primary analysis dataset (Panel A) and for overall survival on the primary analysis dataset (Panel B) and the updated overall survival dataset (Panel C).

A. Progression-free Survival



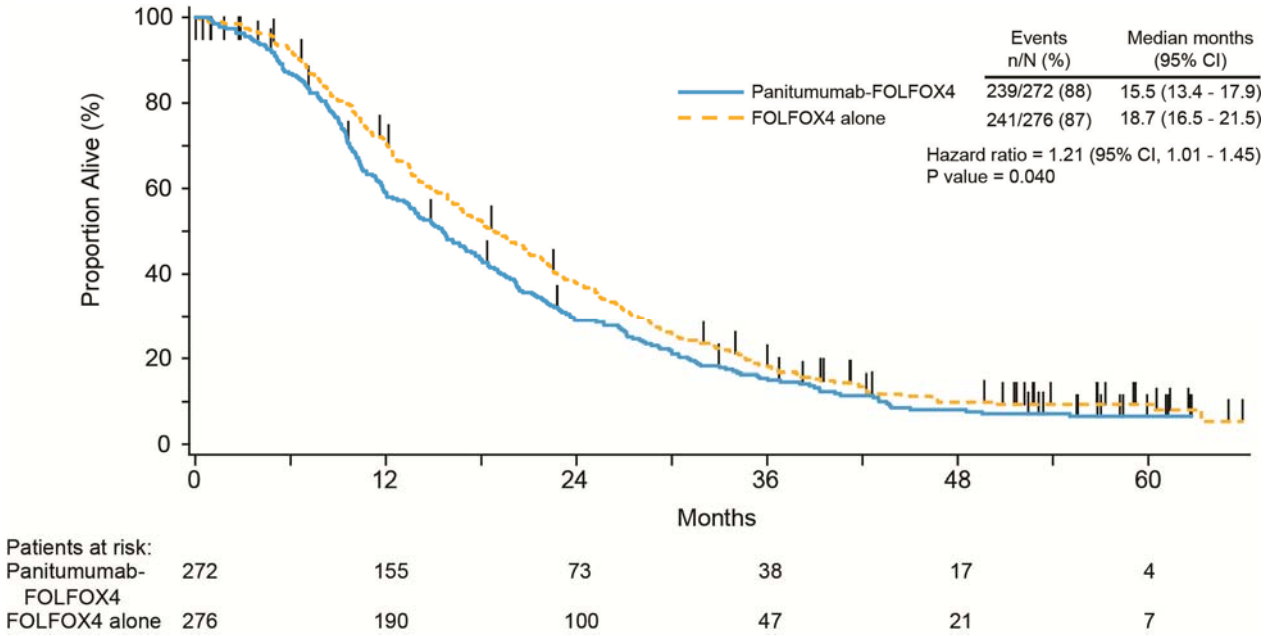
Patients at risk:				
Panitumumab-FOLFOX4	272	144	28	7
FOLFOX4 alone	276	167	43	4

B. Overall Survival (Primary Analysis Dataset)



Patients at risk:			
Panitumumab-FOLFOX4	272	154	48
FOLFOX4 alone	276	187	63

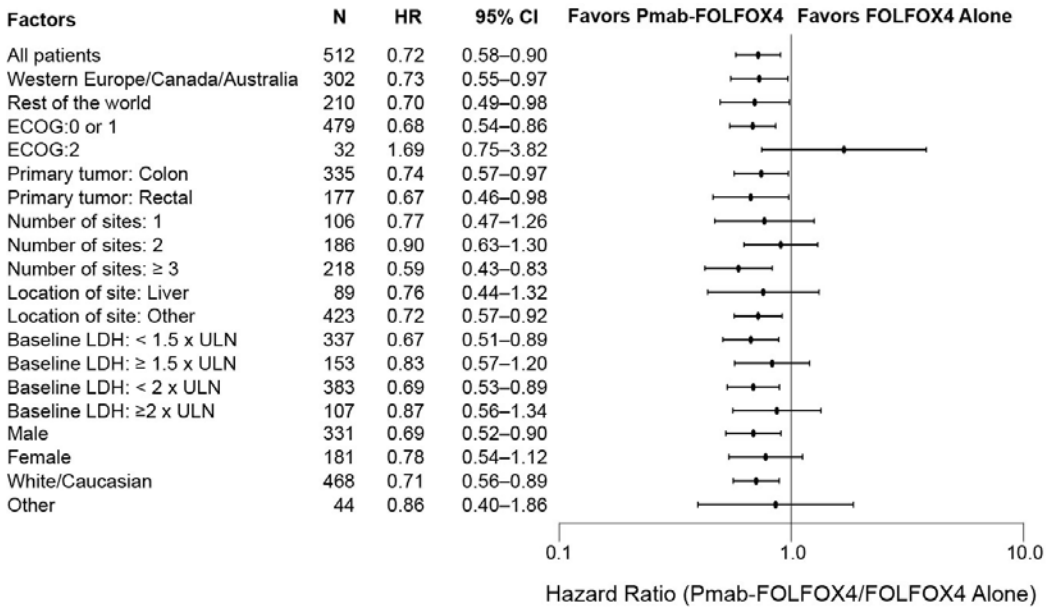
C. Overall Survival (Updated Overall Survival Analysis Dataset)



Patients at risk:						
Panitumumab-FOLFOX4	272	155	73	38	17	4
FOLFOX4 alone	276	190	100	47	21	7

Figure S4. Nonmutated *RAS* Forest Plots of Treatment Hazard Ratios (HRs) with 95% Confidence Intervals (CIs) for Progression-Free Survival (Panel A) and Overall Survival (Panel B) Within Subpopulations (Primary Analysis Dataset).

A. Progression-free Survival



B. Overall Survival

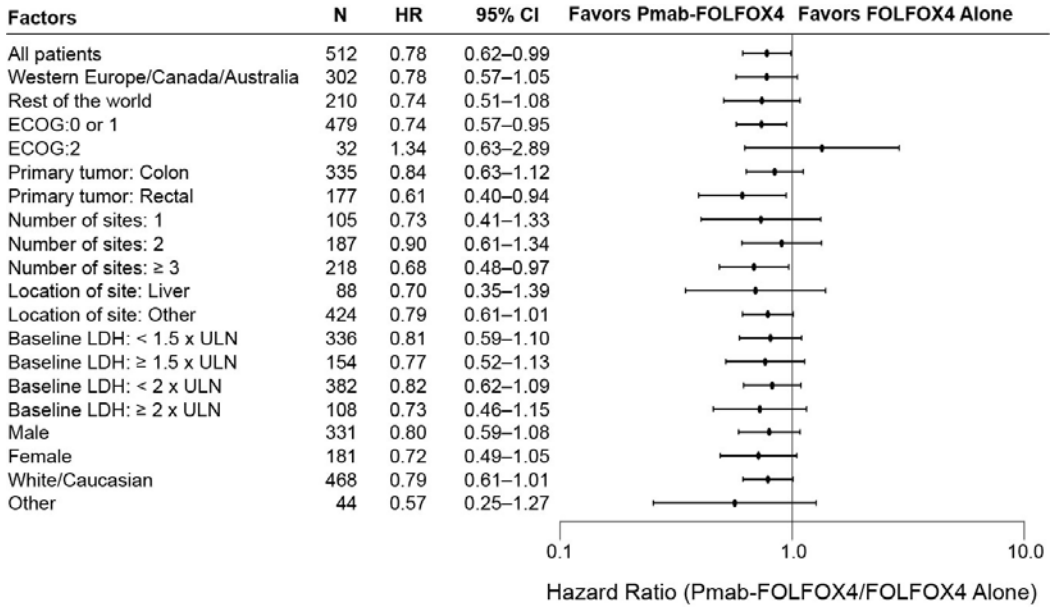


Figure S5. Overall Survival Kaplan-Meier Plot in Patients With Nonmutated *RAS* Excluding Mutated Codon 59 Alleles (Exploratory Analysis of the Primary Analysis Dataset). NE denotes not estimable.

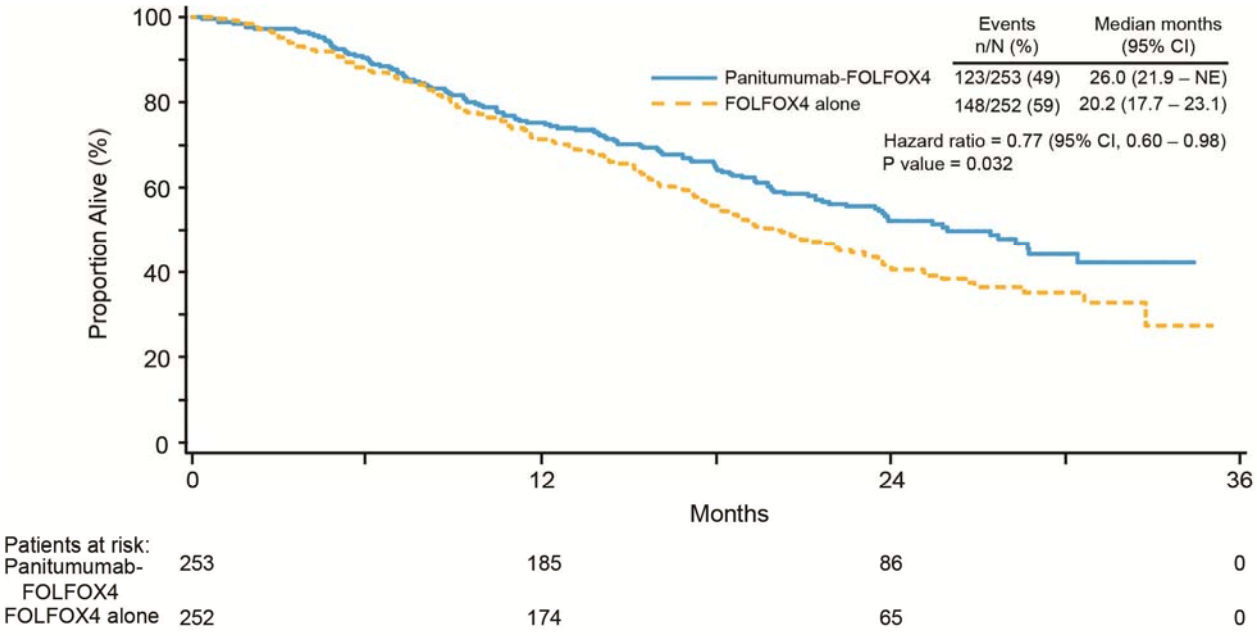
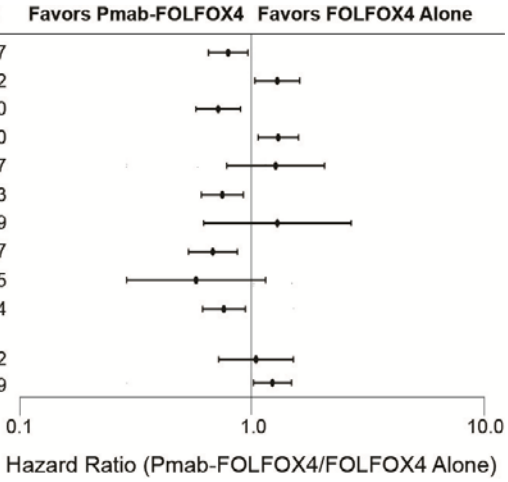


Figure S6. Forest Plots of Treatment Hazard Ratios (HRs) With 95% Confidence Intervals (CIs) for Progression-Free Survival (Panel A) and Overall Survival (Panel B) in *RAS* and *BRAF* subsets (Primary Analysis Dataset). Various nonmutated (wild-type [WT]) and mutated (MT) subsets are shown.

A. Progression-free Survival

Efficacy Analysis Set	N	HR	95% CI
WT *KRAS* exon 2	656	0.80	0.65–0.97
MT *KRAS* exon 2	440	1.30	1.04–1.62
WT *RAS*	512	0.72	0.58–0.90
MT *RAS*	548	1.31	1.07–1.60
WT *KRAS* exon 2, MT other *RAS*	108	1.28	0.79–2.07
WT *KRAS* exon 2, WT *NRAS*	579	0.75	0.61–0.93
WT *KRAS* exon 2, MT *NRAS*	48	1.30	0.63–2.69
WT *RAS*, WT *BRAF*	446	0.68	0.54–0.87
WT *RAS*, MT *BRAF*	53	0.58	0.29–1.15
WT *KRAS* exon 2, WT *BRAF*	566	0.76	0.62–0.94
WT *KRAS* exon 2, MT other *RAS* or MT *BRAF*	161	1.05	0.72–1.52
MT *RAS* or MT *BRAF*	601	1.24	1.02–1.49



B. Overall Survival

Efficacy Analysis Set	N	HR	95% CI
WT *KRAS* exon 2	656	0.83	0.67–1.02
MT *KRAS* exon 2	440	1.25	0.99–1.57
WT *RAS*	512	0.78	0.62–0.99
MT *RAS*	548	1.25	1.02–1.55
WT *KRAS* exon 2, MT other *RAS*	108	1.29	0.79–2.10
WT *KRAS* exon 2, WT *NRAS*	579	0.81	0.65–1.01
WT *KRAS* exon 2, MT *NRAS*	48	1.17	0.56–2.43
WT *RAS*, WT *BRAF*	446	0.74	0.57–0.96
WT *RAS*, MT *BRAF*	53	0.90	0.46–1.76
WT *KRAS* exon 2, WT *BRAF*	566	0.80	0.64–1.00
WT *KRAS* exon 2, MT other *RAS* or MT *BRAF*	161	1.14	0.78–1.66
MT *RAS* or MT *BRAF*	601	1.21	0.99–1.47

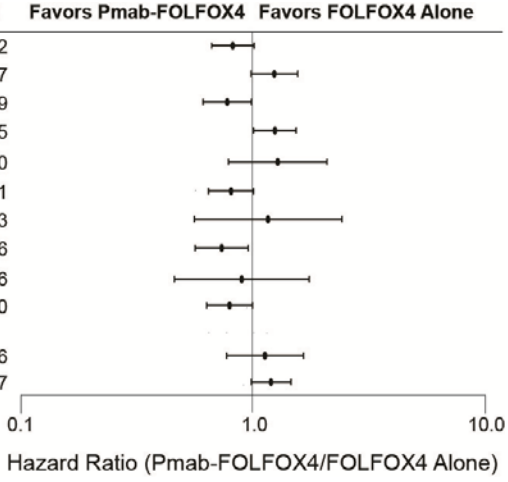


Figure S7. Forest Plots of Treatment Hazard Ratios (HRs) With 95% Confidence Intervals (CIs) of the Panitumumab (Pmab)-FOLFOX4 and FOLFOX4 Alone Arms for Overall Survival (Primary Analysis Dataset).

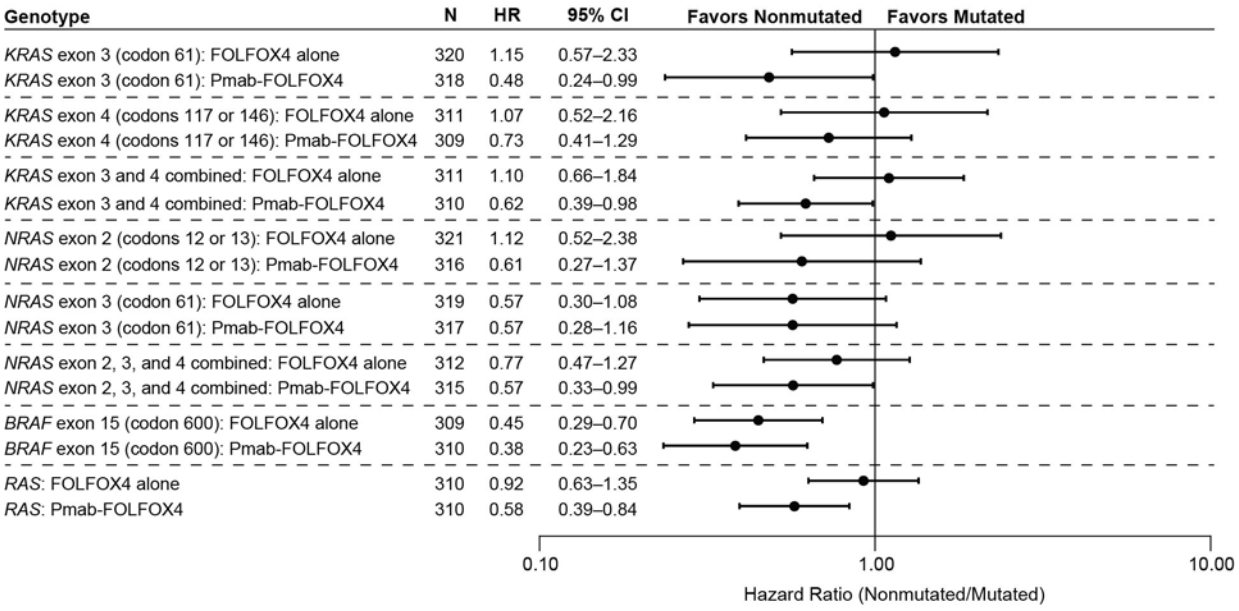


Table S1. Mutations Identified in Available Patient Tumor Specimens*

Patient	*KRAS* exon 2	*KRAS* exon 3	*KRAS* exon 4	*NRAS* exon 2	*NRAS* exon 3	*NRAS* exon 4	*BRAF*
1	WT	WT	WT	WT	WT	WT	WT
2	WT	WT	WT	WT	WT	WT	WT
3	WT	WT	WT	WT	WT	WT	WT
4	WT	WT	WT	WT	WT	WT	WT
5	WT	WT	WT	WT	WT	WT	WT
6	WT	WT	WT	WT	WT	WT	WT
7	WT	WT	WT	WT	WT	WT	WT
8	WT	WT	WT	**G12D**	WT	WT	WT
9	WT	WT	WT	WT	WT	WT	WT
10	WT	WT	WT	WT	WT	WT	WT
11	WT	WT	WT	WT	WT	WT	WT
12	WT	WT	WT	**G13R**	WT	WT	WT
13	WT	WT	WT	WT	WT	WT	WT
14	WT	WT	WT	WT	WT	WT	WT
15	WT	WT	WT	WT	WT	WT	WT
16	WT	WT	WT	WT	WT	WT	WT
17	WT	WT	WT	WT	WT	WT	WT
18	WT	WT	WT	WT	WT	WT	WT
19	WT	WT	WT	WT	WT	WT	WT
20	WT	WT	WT	WT	WT	WT	WT
21	WT	WT	WT	WT	WT	WT	WT
22	WT	WT	WT	WT	WT	WT	WT
23	WT	WT	WT	WT	**Q61K**	WT	WT
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55	WT	WT	WT	**G13R**	WT	WT	WT
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155	WT	WT	WT	WT	WT	WT	**V600E**
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300	WT	FAIL	FAIL	FAIL	FAIL	FAIL	FAIL
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312	WT	WT	WT	WT	WT	WT	**V600E**
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316	WT	WT	**A146T**	WT	WT	WT	WT
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323	WT	WT	WT	WT	WT	WT	**V600E**
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326	WT	WT	**A146T**	WT	WT	WT	WT
327	WT	WT	WT	**G12V**	WT	WT	WT
328	WT	WT	WT	WT	WT	WT	WT
329	WT	WT	WT	WT	WT	WT	**V600E**
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331	WT	**Q61L**	WT	WT	WT	WT	WT
332	WT	WT	WT	WT	WT	WT	WT
333	WT	WT	WT	WT	WT	WT	**V600E**
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341	WT	WT	WT	WT	WT	WT	WT

342	WT	WT	WT	WT	WT	WT	WT
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372	WT	WT	WT	WT	WT	WT	WT
373	WT	WT	WT	WT	WT	WT	**V600E**
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376	WT	WT	FAIL	WT	WT	WT	WT
377†	WT	**Q61H, Q61R**	WT	WT	WT	WT	WT
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388	WT	WT	WT	WT	WT	WT	FAIL

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398	WT	WT	WT	WT	WT	WT	**V600E**
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438	WT	WT	WT	WT	WT	WT	**V600E**
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453	WT	WT	WT	WT	**Q61K**	WT	WT
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455	WT	WT	WT	WT	WT	WT	**V600E**
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470	WT	WT	WT	**G12V**	WT	WT	WT
471	WT	WT	WT	WT	WT	WT	WT
472	WT	WT	WT	WT	WT	WT	WT
473	WT	**Q61H**	WT	WT	FAIL	WT	WT
474	WT	WT	WT	WT	WT	WT	WT
475	WT	WT	WT	WT	WT	WT	WT
476	WT	WT	WT	WT	WT	WT	WT
477	WT	WT	WT	WT	WT	WT	WT
478	WT	WT	WT	WT	WT	WT	WT
479	WT	WT	WT	WT	WT	WT	WT
480	WT	WT	WT	WT	WT	WT	**V600E**
481	WT	WT	WT	WT	WT	WT	WT
482	WT	WT	WT	WT	WT	WT	WT
483	WT	WT	WT	WT	WT	WT	WT
484	WT	WT	WT	WT	WT	WT	WT

485	WT	WT	WT	WT	WT	WT	WT
486	WT	WT	WT	WT	WT	WT	WT
487	WT	WT	WT	**G12D**	WT	WT	WT
488	WT	WT	WT	WT	WT	WT	WT
489	WT	WT	WT	WT	WT	WT	WT
490	WT	WT	WT	WT	WT	WT	WT
491	WT	WT	WT	WT	WT	WT	WT
492	WT	WT	WT	WT	WT	WT	WT
493	WT	WT	WT	WT	WT	WT	WT
494	WT	WT	WT	WT	WT	WT	WT
495	WT	WT	WT	WT	WT	WT	**V600E**
496	WT	WT	WT	WT	WT	WT	WT
497	WT	WT	WT	WT	WT	WT	WT
498	WT	WT	WT	WT	WT	WT	WT
499	WT	WT	WT	WT	WT	WT	WT
500	WT	WT	WT	WT	WT	WT	**V600E**
501	WT	WT	WT	WT	**Q61K**	WT	WT
502	WT	WT	WT	WT	WT	WT	WT
503	WT	WT	WT	WT	WT	WT	WT
504	WT	WT	WT	WT	WT	WT	WT
505	WT	WT	WT	WT	WT	WT	WT
506	WT	WT	WT	**G13R**	WT	WT	WT
507	WT	WT	WT	WT	WT	WT	**V600E**
508	WT	WT	WT	WT	WT	WT	WT
509	WT	WT	WT	WT	WT	WT	WT
510	WT	WT	WT	WT	WT	WT	WT
511	WT	WT	WT	WT	WT	WT	**V600E**
512	WT	WT	WT	WT	WT	WT	FAIL
513	WT	WT	WT	WT	WT	WT	WT
514	WT	WT	WT	WT	WT	WT	**V600E**
515	WT	WT	WT	WT	WT	WT	WT
516	WT	WT	WT	WT	WT	WT	WT
517	WT	WT	WT	WT	**Q61K**	WT	WT
518	WT	WT	WT	WT	WT	WT	WT
519	WT	WT	WT	WT	WT	WT	WT
520	WT	WT	WT	WT	WT	WT	WT
521	WT	WT	WT	WT	WT	WT	WT
522	WT	WT	WT	WT	WT	WT	WT
523	WT	WT	WT	WT	WT	WT	WT
524	WT	WT	WT	WT	WT	WT	WT
525	WT	WT	WT	WT	**Q61R**	WT	WT
526	WT	WT	WT	WT	WT	WT	WT
527	WT	WT	WT	WT	WT	WT	WT
528	WT	WT	WT	WT	WT	WT	WT
529	WT	WT	WT	WT	WT	WT	WT
530	WT	WT	WT	WT	WT	WT	WT
531	WT	WT	WT	WT	WT	WT	WT
532	WT	WT	WT	WT	WT	WT	**V600E**
533	WT	WT	WT	WT	WT	WT	WT
534	WT	WT	WT	WT	WT	WT	WT

535	WT	WT	WT	WT	WT	WT	WT
536	WT	WT	WT	WT	WT	WT	**V600E**
537	WT	WT	WT	WT	WT	WT	WT
538	WT	WT	WT	WT	WT	WT	WT
539	WT	WT	WT	WT	WT	WT	WT
540	WT	WT	WT	**G12C**	WT	WT	WT
541	WT	WT	WT	WT	WT	WT	WT
542	WT	WT	WT	WT	WT	WT	WT
543	WT	WT	WT	WT	WT	WT	WT
544	WT	WT	WT	WT	WT	WT	WT
545	WT	WT	WT	WT	WT	WT	WT
546	WT	WT	**A146T**	WT	WT	WT	WT
547	WT	WT	WT	WT	WT	WT	WT
548	WT	WT	WT	WT	WT	WT	WT
549	WT	WT	WT	WT	WT	WT	WT
550	WT	WT	WT	WT	WT	WT	WT
551	WT	WT	WT	WT	WT	WT	WT
552	WT	WT	WT	WT	WT	WT	WT
553	WT	WT	WT	WT	WT	WT	WT
554	WT	WT	WT	WT	WT	WT	**V600E**
555	WT	WT	WT	WT	WT	WT	WT
556	WT	WT	WT	WT	WT	WT	WT
557	WT	WT	WT	WT	WT	WT	WT
558	WT	WT	WT	WT	WT	FAIL	WT
559	WT	WT	**A146T**	WT	WT	WT	WT
560	WT	WT	WT	WT	WT	WT	**V600E**
561	WT	WT	WT	WT	WT	WT	WT
562	WT	WT	FAIL	WT	WT	WT	WT
563	WT	WT	WT	WT	WT	WT	WT
564	WT	WT	**K117N**	WT	WT	WT	WT
565	WT	WT	WT	WT	WT	WT	WT
566	WT	WT	WT	WT	WT	WT	FAIL
567	WT	WT	WT	WT	WT	WT	WT
568	WT	WT	WT	WT	**Q61R**	WT	WT
569	WT	WT	WT	WT	WT	WT	WT
570	WT	WT	FAIL	WT	WT	WT	WT
571	WT	WT	WT	WT	WT	WT	WT
572	WT	WT	WT	WT	WT	WT	**V600E**
573	WT	FAIL	FAIL	WT	WT	FAIL	FAIL
574	WT	WT	WT	WT	WT	WT	WT
575	WT	WT	WT	WT	WT	WT	WT
576	WT	WT	WT	**G12D**	FAIL	WT	FAIL
577	WT	WT	WT	WT	WT	WT	WT
578	WT	WT	**K117N**	WT	WT	WT	WT
579	WT	**Q61R**	WT	WT	WT	WT	WT
580	WT	WT	WT	WT	WT	WT	WT
581	WT	WT	WT	WT	WT	WT	WT
582	WT	WT	WT	WT	WT	WT	**V600E**
583	WT	WT	WT	WT	WT	WT	WT
584	WT	WT	WT	WT	WT	WT	WT

585	WT	WT	WT	WT	WT	WT	WT
586	WT	WT	WT	WT	WT	WT	WT
587	WT	WT	WT	WT	WT	WT	WT
588	WT	WT	WT	WT	WT	WT	WT
589	WT	WT	WT	WT	WT	WT	WT
590	WT	WT	WT	WT	WT	WT	FAIL
591	WT	WT	WT	WT	WT	WT	WT
592	WT	WT	WT	WT	WT	WT	**V600E**
593	WT	WT	WT	WT	WT	WT	WT
594	WT	WT	WT	WT	WT	WT	WT
595	WT	WT	FAIL	WT	WT	FAIL	FAIL
596	WT	WT	FAIL	WT	WT	FAIL	FAIL
597	WT	WT	WT	WT	WT	WT	WT
598	WT	WT	WT	WT	WT	WT	WT
599	WT	WT	WT	WT	WT	WT	WT
600	WT	WT	WT	WT	**Q61H**	WT	WT
601	WT	WT	WT	WT	WT	WT	WT
602	WT	WT	WT	WT	WT	WT	WT
603	WT	WT	WT	WT	WT	WT	WT
604	WT	WT	**A146T**	WT	WT	WT	WT
605	WT	WT	WT	WT	WT	WT	WT
606	WT	WT	WT	WT	WT	WT	FAIL
607	WT	WT	WT	WT	WT	WT	WT
608	WT	WT	WT	WT	WT	WT	**V600E**
609	WT	WT	WT	WT	WT	WT	WT
610	WT	WT	**A146T**	WT	WT	WT	WT
611	WT	WT	WT	WT	WT	WT	**V600E**
612	WT	WT	WT	WT	WT	WT	WT
613	WT	WT	WT	WT	WT	WT	**V600E**
614	WT	WT	WT	WT	WT	WT	WT
615	WT	WT	**A146V**	WT	WT	WT	WT
616	WT	WT	WT	WT	WT	WT	WT
617	WT	WT	WT	WT	WT	WT	**V600E**
618	WT	WT	WT	WT	WT	WT	WT
619	WT	WT	WT	WT	WT	WT	WT
620	WT	WT	WT	WT	WT	WT	WT
621	WT	WT	WT	WT	WT	WT	FAIL
622	WT	WT	WT	WT	WT	WT	WT
623	WT	WT	WT	WT	WT	WT	WT
624	WT	WT	WT	WT	WT	WT	WT
625	WT	WT	**A146P**	WT	WT	WT	WT
626	WT	WT	WT	WT	WT	WT	WT
627	WT	WT	WT	WT	WT	WT	WT
628	WT	WT	WT	WT	WT	WT	WT
629	WT	WT	WT	WT	WT	WT	WT
630	WT	WT	**A146V**	WT	WT	WT	WT
631	WT	WT	WT	WT	WT	WT	WT
632	WT	WT	WT	WT	WT	WT	WT
633	WT	WT	WT	WT	WT	WT	WT
634	WT	WT	WT	WT	WT	WT	WT

635	WT	WT	WT	WT	WT	WT	WT
636	WT	WT	WT	WT	WT	WT	WT
637	WT	WT	WT	WT	WT	WT	WT
638	WT	WT	WT	WT	WT	WT	WT
639	WT	WT	FAIL	FAIL	WT	FAIL	FAIL
640	WT	WT	WT	WT	WT	WT	WT
641	WT	WT	WT	WT	WT	WT	WT

WT denotes wild-type (nonmutated)

*All *RAS* exons in a patient sample had to yield a wild-type result to be characterized as nonmutated *RAS*. Patient samples that had any *RAS* exon mutation, regardless of whether other *RAS* exons failed to yield a result, were characterized as mutated *RAS.*

†Due to presumed tumor heterogeneity.

Table S2. Efficacy Results by Unevaluable Biomarker Status (Primary Analysis Dataset)

	Pmab-FOLFOX4	FOLFOX4 Alone	HR (95% CI)	P value
Unevaluable *RAS* - n	62	61		
Median PFS - mos (95% CI)				
Primary	7.9 (7.2 - 9.9)	8.9 (6.6 - 10.4)	0.83 (0.51 - 1.35)	0.454
Median OS - mos (95% CI)				
Primary	18.5 (13.7 - 28.6)	14.3 (11.7 - 17.6)	0.74 (0.46 - 1.20)	0.218
Unevaluable *RAS* and *BRAF* - n	69	67		
Median PFS - mos (95% CI)				
Primary	9.2 (7.2 - 12.6)	9.4 (7.4 - 11.3)	1.02 (0.65 - 1.59)	0.922
Median OS - mos (95% CI)				
Primary	18.5 (13.9 - 22.9)	15.5 (12.4 - 24.3)	0.93 (0.59 - 1.46)	0.758

Pmab denotes panitumumab, PFS progression-free survival, OS overall survival, HR hazard ratio, mos months, CI confidence interval

Table S3. Efficacy Results for Nonmutated *RAS* and Nonmutated *RAS* Excluding Mutated Codon 59 Alleles (Exploratory Analysis of the Primary Analysis Dataset).

	Pmab-FOLFOX4	FOLFOX4 Alone	HR (95% CI)	P Value
Nonmutated *RAS* - n	259	253		
Median PFS - mos (95% CI)	10.1 (9.3 - 12.0)	7.9 (7.2 - 9.3)	0.72 (0.58 - 0.90)	0.004
Median OS - mos (95% CI)	26.0 (21.7 - 30.4)	20.2 (17.7 - 23.1)	0.78 (0.62 - 0.99)	0.043
Nonmutated *RAS* (excluding codon 59 mutated alleles) - n	253	252		
Median PFS - mos (95% CI)	10.4 (9.3 - 12.1)	7.9 (7.2 - 9.3)	0.71 (0.57 - 0.89)	0.002
Median OS - mos (95% CI)	26.0 (21.9 - NE)	20.2 (17.7 - 23.1)	0.77 (0.60 - 0.98)	0.032

Pmab denotes panitumumab, PFS progression-free survival, OS overall survival, NE not estimable, HR hazard ratio, mos months, CI confidence interval