

ORIGINAL ARTICLE

Panitumumab–FOLFOX4 Treatment and RAS Mutations in Colorectal Cancer

Jean-Yves Douillard, M.D., Ph.D., Kelly S. Oliner, Ph.D., Salvatore Siena, M.D., Josep Tabernero, M.D., Ronald Burkes, M.D., Mario Barugel, M.D., Yves Humblet, M.D., Ph.D., Gyorgy Bodoky, M.D., Ph.D., David Cunningham, M.D., Jacek Jassem, M.D., Ph.D., Fernando Rivera, M.D., Ph.D., Ilona Kocáková, M.D., Ph.D., Paul Ruff, M.D., Maria Błasińska-Morawiec, M.D., Martin Šmakal, M.D., Jean Luc Canon, M.D., Mark Rother, M.D., Richard Williams, M.B., B.S., Ph.D., Alan Rong, Ph.D., Jeffrey Wizeorek, M.D., Roger Sidhu, M.D., and Scott D. Patterson, Ph.D.

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 non-mutated *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.)

From Institut de Cancérologie de l'Ouest (ICO) René Gauducheau, Nantes, France (J.-Y.D.); Amgen, Thousand Oaks, CA (K.S.O., R.W., A.R., J.W., R.S., S.D.P.); Ospedale Niguarda Ca' Granda, Milan (S.S.); Vall d'Hebron University Hospital, Universitat Autònoma de Barcelona, Barcelona (J.T.), and Hospital Universitario Marqués de Valdecilla, Santander (F.R.) — both in Spain; Mount Sinai Hospital, Toronto (R.B.), and Credit Valley Hospital, Mississauga, ON (M.R.) — both in Canada; Hospital de Gastroenterología, Buenos Aires (M.B.); Université Catholique de Louvain, Brussels (Y.H.), and Grand Hôpital de Charleroi, Charleroi (J.L.C.) — both in Belgium; Szent László Hospital, Budapest, Hungary (G.B.); Royal Marsden National Health Service Foundation Trust, London (D.C.); Medical University of Gdansk, Gdansk (J.J.), and Copernicus Memorial Hospital, Lodz (M.B.-M.) — both in Poland; Masaryk Memorial Cancer Institute, Brno (I.K.), and Institute of Oncology and Rehabilitation Pleš, Nová Ves pod Pleš (M.Š.) — both in the Czech Republic; and the University of the Witwatersrand Faculty of Health Sciences, Johannesburg (P.R.). Address reprint requests to Dr. Douillard at ICO René Gauducheau, Blvd. Jacques Monod, 44805 Saint Herblain, France, or at jean-yves.douillard@ico.unicancer.fr.

Drs. Douillard and Oliner contributed equally to this article.

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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 monotherapy¹⁰ 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
<i>KRAS</i> exon 2 at codons 12 and 13 — no. of patients			
Nonmutated	325	331	656
Mutated†	221	219	440
<i>KRAS</i> exon 2 tumors tested for <i>RAS</i> and <i>BRAF</i> — no./total no. (%)‡			
<i>KRAS</i> 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)
<i>KRAS</i> 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)
<i>NRAS</i> 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)
<i>NRAS</i> 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)
<i>NRAS</i> 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)
<i>BRAF</i> 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. (%)			
<i>RAS</i>	531/593 (90)	529/590 (90)	1060/1183 (90)§
<i>BRAF</i>	310/593 (52)	309/590 (52)	619/1183 (52)
<i>RAS</i> and <i>BRAF</i>	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.

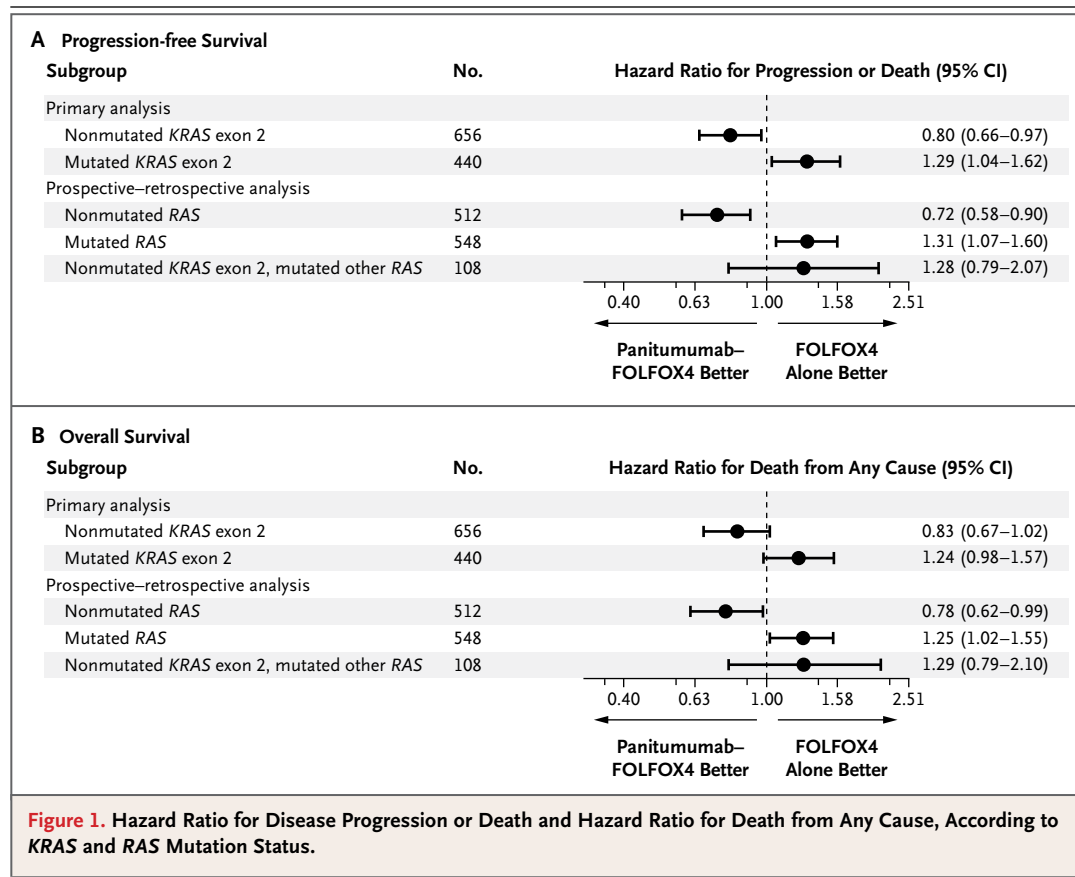
Table 2. Efficacy Results According to RAS Mutation Status.

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 up-

dated 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



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.

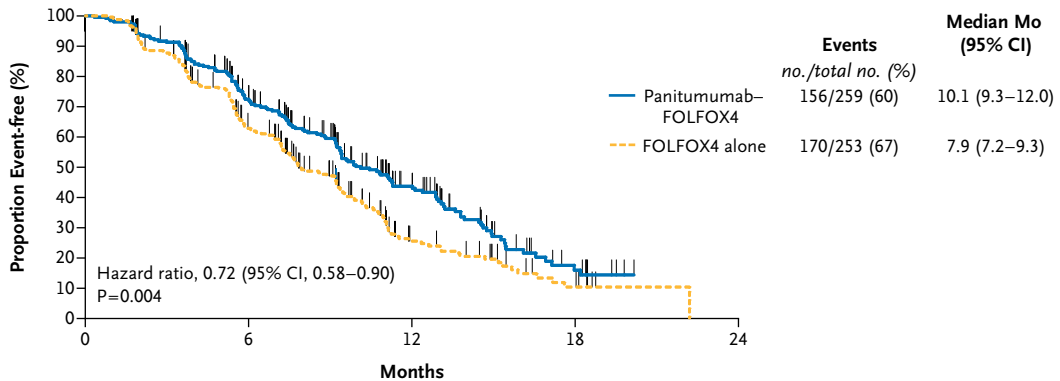
The treatment effect on progression-free survival and overall survival in favor of panitumumab–FOLFOX4 in patients with nonmutated *RAS* was observed across subpopulations predefined accord-

ing to baseline covariates, except an ECOG performance-status score of 2 (Fig. S4 in the Supplementary Appendix).

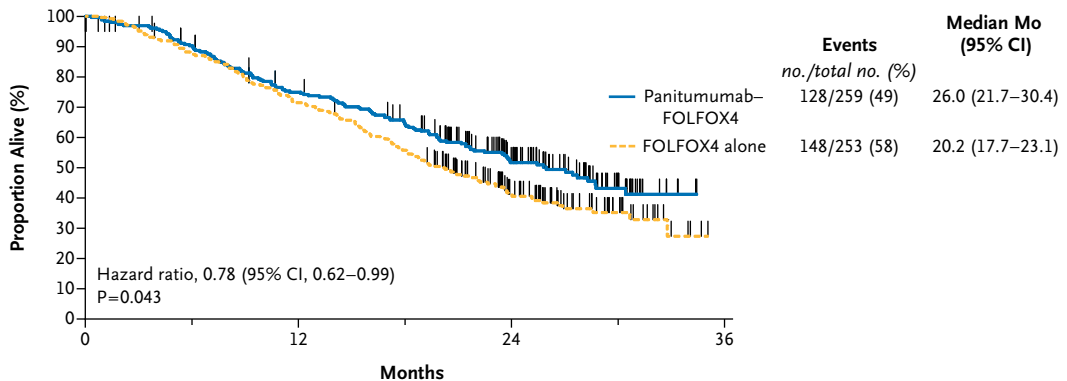
Subsequent to the prespecified analysis, previously reported mutations in *KRAS* and *NRAS* at codon 59 (A59G and A59T)^{9,28–30} were identified in seven patients. In an exploratory analysis involving this small patient population, exclusion of these mutated alleles slightly improved progression-free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

EFFICACY ACCORDING TO TUMOR *BRAF* STATUS

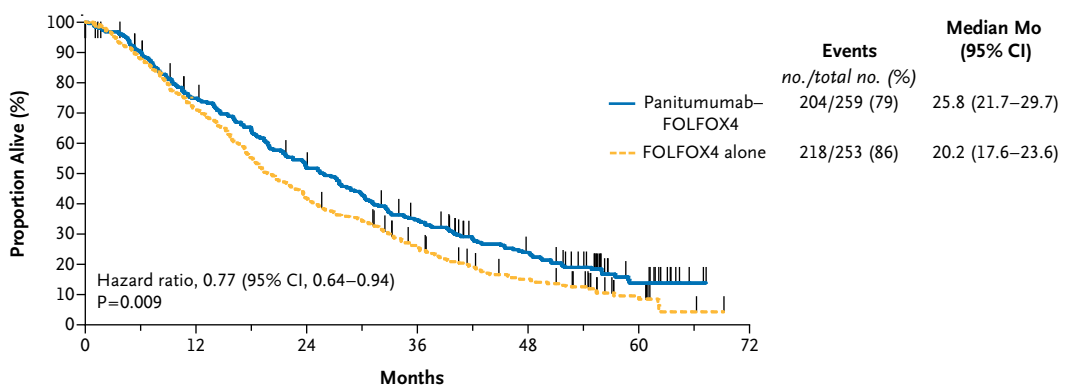
In the nonmutated *RAS* and nonmutated *BRAF* subgroup, panitumumab–FOLFOX4 was associated with a 1.6-month improvement in progression-free survival and a 7.4-month improvement in overall survival, as compared with FOLFOX4 alone (Table 3). The minor differences between FOLFOX4–panitumumab and FOLFOX4 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**No. at Risk**

Panitumumab-FOLFOX4	259	171	65	10
FOLFOX4 alone	253	140	31	7

B Overall Survival in the Primary-Analysis Population**No. at Risk**

Panitumumab-FOLFOX4	259	189	88	0
FOLFOX4 alone	253	174	65	0

C Overall Survival in the Updated-Analysis Population**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)
	<i>number of patients (percent)</i>					
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 per- manent 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 2² 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 muta-

tions 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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