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JAMA. 2010;304(16):1812-1820 (doi:10.1001/jama.2010.1535)

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Association of *KRAS* p.G13D Mutation With Outcome in Patients With Chemotherapy-Refractory Metastatic Colorectal Cancer Treated With Cetuximab

Wendy De Roock, MD
Derek J. Jonker, MD
Federica Di Nicolantonio, PhD
Andrea Sartore-Bianchi, MD
Dongsheng Tu, PhD
Salvatore Siena, MD
Simona Lamba, MSc
Sabrina Arena, PhD
Milo Frattini, PhD
Hubert Piessevaux, MD, PhD
Eric Van Cutsem, MD, PhD
Chris J. O'Callaghan, DVM, PhD
Shirin Khambata-Ford, PhD
John R. Zalcberg, MBBS, PhD
John Simes, MD
Christos S. Karapetis, MD
Alberto Bardelli, PhD
Sabine Tejpar, MD, PhD

Context Patients with metastatic colorectal cancer who have *KRAS* codon 12– or *KRAS* codon 13–mutated tumors are presently excluded from treatment with the anti–epidermal growth factor receptor monoclonal antibody cetuximab.

Objective To test the hypothesis that *KRAS* codon 13 mutations are associated with a better outcome after treatment with cetuximab than observed with other *KRAS* mutations.

Design, Setting, and Patients We studied the association between *KRAS* mutation status (p.G13D vs other *KRAS* mutations) and response and survival in a pooled data set of 579 patients with chemotherapy-refractory colorectal cancer treated with cetuximab between 2001 and 2008. Patients were included in the CO.17, BOND, MABEL, EMR202600, EVEREST, BABEL, or SALVAGE clinical trials or received off-study treatment. Univariate and multivariate analyses, adjusting for possible prognostic factors and data set, were performed. The effect of the different mutations was studied in vitro by constructing isogenic cell lines with wild-type *KRAS*, p.G12V, or p.G13D mutant alleles and treating them with cetuximab.

Main Outcome Measures The main efficacy end point was overall survival. Secondary efficacy end points were response rate and progression-free survival.

Results In comparison with patients with other *KRAS*-mutated tumors, patients with p.G13D-mutated tumors (n=32) treated with cetuximab had longer overall survival (median, 7.6 [95% confidence interval {CI}, 5.7-20.5] months vs 5.7 [95% CI, 4.9-6.8] months; adjusted hazard ratio [HR], 0.50; 95% CI, 0.31-0.81; $P=.005$) and longer progression-free survival (median, 4.0 [95% CI, 1.9-6.2] months vs 1.9 [95% CI, 1.8-2.8] months; adjusted HR, 0.51; 95% CI, 0.32-0.81; $P=.004$). There was a significant interaction between *KRAS* mutation status (p.G13D vs other *KRAS* mutations) and overall survival benefit with cetuximab treatment (adjusted HR, 0.30; 95% CI, 0.14-0.67; $P=.003$). In vitro and mouse model analysis showed that although p.G12V-mutated colorectal cells were insensitive to cetuximab, p.G13D-mutated cells were sensitive, as were *KRAS* wild-type cells.

Conclusions In this analysis, use of cetuximab was associated with longer overall and progression-free survival among patients with chemotherapy-refractory colorectal cancer with p.G13D-mutated tumors than with other *KRAS*-mutated tumors. Evaluation of cetuximab therapy in these tumors in prospective randomized trials may be warranted.

JAMA. 2010;304(16):1812-1820 www.jama.com

Recent retrospective correlative analyses of metastatic colorectal cancer trials indicate that patients with *KRAS*-mutated tumors (NCBI Entrez Gene 3845) do not benefit from the anti–epidermal growth factor receptor (EGFR) monoclonal antibodies cetuximab and panitumumab.[1] These retrospective analyses were performed independently, and for each analysis, *KRAS* wild-type vs mutant were studied grouping codons 12 and 13 mutations together, without subgroup analysis. Health authorities in the United States and Europe have indicated that patients with *KRAS* codon 12– or *KRAS* codon 13–mutated tumors should not receive cetuximab or panitumumab.[2-4]

However, indications exist that not all *KRAS* mutations are equal in their biological characteristics. First, the pattern of *KRAS* mutations is tumor-type spe-

Author Affiliations are listed at the end of this article.
Corresponding Authors: Sabine Tejpar, MD, PhD, Digestive Oncology Unit, University Hospital Gasthuisberg, Herestraat 49, B-3000, Leuven, Belgium (sabine.tejpar@uzleuven.be); Alberto Bardelli, PhD, Laboratory of Molecular Genetics, Institute for Cancer Research and Treatment, University of Turin Medical School, Strada Provinciale 142, Km 3.95, 10060 Candiolo, Turin, Italy (a.bardelli@unito.it).

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cific. Although colorectal tumors have both codon 12 and codon 13 mutations (79% and 17.6%, respectively), *KRAS*-mutated pancreatic tumors (75%-95%) almost invariably carry codon 12 mutations, and in non–small cell lung cancer, more than 90% of *KRAS* mutations are located in codon 12.[5] Second, anecdotal reports indicate that a minority of patients (<10%) with *KRAS*-mutated tumors can respond to anti-EGFR therapy[6-9] and that about 15% have long-term disease stabilization.[10] In these patients' tumors, codon 13 mutations were overrepresented compared with the overall *KRAS*-mutated tumor population. Finally, *KRAS* codon 13 mutations exhibit weaker in vitro transforming activity than codon 12 mutations.[11]

Based on these observations, we hypothesized that *KRAS* codon 13 mutations may be associated with a better outcome after cetuximab treatment than observed with other *KRAS* mutations. Because the glycine (G)-to-aspartate (D) transition mutation is the most frequent codon 13 mutation in colorectal cancer,[5] we studied the association of this p.G13D mutation with outcome after cetuximab treatment in a pooled data set of 579 patients with chemotherapy-refractory metastatic colorectal cancer treated with cetuximab with or without chemotherapy.

METHODS

Description of Data Sets

All patients included had chemotherapy-refractory metastatic colorectal cancer. The National Cancer Institute of Canada Clinical Trials Group/Australasian Gastrointestinal Trials Group (NCIC CTG/AGITG) data set is from the CO.17 randomized phase 3 trial of cetuximab plus best supportive care vs best supportive care alone.[12] *KRAS* mutation analysis was performed by direct sequencing as described previously.[13]

All centers participating in the NCIC CTG CO.17 trial received approval from their local research ethics body for participation in the trial, including for collection of tissue for correlative biomarker analyses, and all NCIC CTG/AGITG patients provided written informed consent for the original clinical trial. The Leuven data set comprises patients from 4 clinical trials (EVEREST, BOND, SALVAGE, and BABEL).[14-16] *KRAS* mutation analysis was performed by allelic discrimination assay as described previously.[10] The Italian data set comprises patients treated at the Ospedale Niguarda Ca'Granda in Milan, Italy, and the Ospedale San Giovanni in Bellinzona, Cantone Ticino, Switzerland. Patients were included in 3 clinical trials (BOND, MABEL, and EMR202600)[15,17,18] or were considered suitable to receive a subsequent line of treatment off study (after becoming refractory to the standard chemotherapy lines). *KRAS* mutation analysis was performed by direct sequencing as described previously.[6-8]

All patients in the Leuven and Italian data sets provided written informed consent to the original clinical trial and to molecular analyses. The Italian data set had a significantly higher percentage of patients with *KRAS* wild-type tumors than the other data sets ($P<.001$) because of the early introduction of *KRAS* testing in Italy, leading to exclusion of patients with *KRAS*-mutated tumors from treatment with cetuximab. A detailed breakdown accounting for the number of participants with each *KRAS* mutation type and patient characteristics is presented for each data set in the eTable (available online at http://www.jama.com).

End Points

The main efficacy end point was overall survival, defined as time from randomization in the NCIC CTG/AGITG data set, from start of cetuximab in the Leuven data set, and from start of cetuximab or date of randomization in the Italian data set to death due to any cause or to last known

Table 1. Patient Characteristics According to *KRAS* Mutation Status[a]

Characteristics	*KRAS* p.G13D Mutation (n = 45)	Other *KRAS* Mutations (n = 265)	*KRAS* Wild-Type (n = 464)	*P* Value[b]
Age, median (range), y	65.0 (39.4-80.0)	62.0 (34.0-89.0)	62.0 (26.0-85.9)	
<65	22 (48.9)	153 (57.7)	278 (59.9)	.80
≥65	23 (51.1)	112 (42.3)	183 (39.4)	
Missing data	0	0	3 (0.6)	
Sex				
Female	18 (40.0)	109 (41.1)	157 (33.8)	.13
Male	27 (60.0)	156 (58.9)	307 (66.2)	
ECOG performance score				
0	10 (22.2)	54 (20.4)	114 (24.6)	.51
1	25 (55.6)	162 (61.1)	255 (55.0)	
2	7 (15.6)	30 (11.3)	48 (10.3)	
Missing data[c]	3 (6.7)	19 (7.2)	47 (10.1)	
Site of primary tumor				
Rectum only	10 (22.2)	55 (20.8)	111 (23.9)	.61
Colon	35 (77.8)	210 (79.2)	352 (75.9)	
Missing data	0	0	1 (0.2)	
Prior chemotherapy regimen[d]				
Fluoropyrimidine (fluorouracil or capecitabine)	45 (100.0)	253 (95.5)	435 (93.8)	.40
Irinotecan	45 (100.0)	258 (97.4)	446 (96.1)	.51
Oxaliplatin	37 (82.2)	233 (87.9)	411 (88.6)	.08
All 3	37 (82.2)	231 (87.2)	402 (86.6)	.20
Treatment				
Cetuximab monotherapy	10 (22.2)	91 (34.3)	146 (31.5)	.48
Cetuximab plus chemotherapy	22 (48.9)	105 (39.6)	205 (44.2)	
No cetuximab	13 (28.9)	69 (26.0)	113 (24.4)	

Abbreviation: ECOG, Eastern Cooperative Oncology Group.
[a] Data are expressed as No. (%) of patients unless otherwise indicated.
[b] *P* values are for comparison of biomarker-positive and -negative groups by χ^2 test for categorical variables and by *t* test for continuous variables.
[c] Excluded in multivariate analyses.
[d] A patient may have received more than 1 prior chemotherapy regimen.

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Table 2. Overall and Progression-Free Survival in Untreated Patients in the CO.17 Trial Only[a]

Survival	*KRAS* Mutation		*KRAS* Wild-Type (n = 464)
	KRAS p.G13D Mutation (n = 45)	Other *KRAS* Mutation (n = 265)	
Overall survival, No.	13	69	113
Median survival (95% CI), mo	3.6 (2.2-4.8)	4.7 (3.6-6.7)	5.0 (4.2-5.5)
Univariate HR (95% CI)	1 [Reference]	1.90 (1.03-3.51)	1.90 (1.05-3.41)
Log-rank *P* value		.04	.03
Multivariate HR (95% CI)	1 [Reference]	1.39 (0.73-2.64)	1.82 (0.99-3.34)
Cox regression *P* value		.33	.053
Progression-free survival, No.	10	91	146
Median survival (95% CI), mo	1.7 (1.5-1.7)	1.8 (1.7-1.9)	1.9 (1.8-2.0)
Univariate HR (95% CI)	1 [Reference]	1.30 (0.71-2.37)	1.35 (0.75-2.43)
Log-rank *P* value		.40	.32
Multivariate HR (95% CI)	1 [Reference]	1.36 (0.73-2.57)	1.31 (0.70-2.43)
Cox regression *P* value		.34	.40

Abbreviations: CI, confidence interval; HR, hazard ratio.
[a]Hazard ratios are expressed for comparison of *KRAS* p.G13D mutation vs other status.

date alive. Secondary efficacy end points were response rate and progression-free survival. Tumor response was evaluated by radiologic imaging every 8 weeks in the NCIC CTG/AGITG data set and every 6 weeks until week 24, 30, or 36 and from then on every 12 weeks in the Leuven data set and for trial patients in the Italian data set. Response Evaluation Criteria in Solid Tumors were used to classify tumor response in all data sets. Progression-free survival was defined as the time from randomization or start of cetuximab to disease progression or death due to any cause. If a patient had not progressed or died at the time of data cutoff, progression-free survival was censored on the date of last disease assessment (NCIC CTG/AGITG data set) or last radiologic assessment (Leuven and Italian data sets).

Statistical Analysis

Differences in response rates by *KRAS* status (p.G13D mutant, other *KRAS* mutant, or *KRAS* wild-type) were evaluated pairwise using the Fisher exact test. Median overall and progression-free survival were estimated by the Kaplan-Meier method and compared between groups using the log-rank test. Hazard ratios (HRs) between groups were estimated from Cox regression models including the following potential prognostic factors: age (<65 vs ≥65 years), sex (male vs female), performance status (Eastern Cooperative Oncology Group performance score, 0-1 vs 2), whether all 3 chemotherapy drugs (fluoropyrimidine [fluorouracil or capecitabine], irinotecan, and oxaliplatin) were previously received (yes vs no), site of primary malignancy (rectum only vs colon), data set (NCIC CTG/AGITG vs Leuven vs Italian), and an interaction term between *KRAS* status and treatment group as indicated.

For the predictive analysis (association between mutation status and outcome after cetuximab treatment), an additional analysis using the CO.17 database only was also undertaken. This was performed as a sensitivity analysis (avoiding the potential bias associated with cross-trial comparisons) rather than as the primary analysis because of the smaller number of patients (n=195). All *P* values were 2-sided and statistical significance was assumed for $P<.05$. No adjustments were made for multiple comparisons. Analyses were carried out using SAS software, version 9 (SAS Institute Inc, Cary, North Carolina).

Measurement of Cetuximab Sensitivity in Isogenic Cell Lines

Details about the generation of *KRAS*-mutated isogenic cells, in vitro and in vivo treatment with cetuximab, proliferation assays, and biochemical profiling of signaling pathways are provided in the eAppendix.

RESULTS

Study Population

Baseline patient characteristics in the 3 data sets by *KRAS* mutation status demonstrated no significant differences (TABLE 1). The mutation frequencies (40% *KRAS*-mutated, of which 14.5% were p.G13D-mutated) and distribution in this pooled data set (eTable) are similar to those reported for randomized trial populations (between 36% and 43% *KRAS*-mutated, of which 15.7% were p.G13D-mutated).[19-22] The objective response rate in unselected patients was 10.2% in the monotherapy group and 22.2% in the cetuximab plus chemotherapy group. The median overall and progression-free survival were, respectively, 7.2 (95% confidence interval [CI], 6.2-8.3) months and 2.3 (95% CI, 1.9-2.9) months in the monotherapy group and 9.2 (95% CI, 8.7-10.1) months and 4.1 (95% CI, 3.9-4.8) months in the cetuximab plus chemotherapy group, comparable with outcomes from randomized trials.[12,15]

Association of p.G13D Mutation With Outcome in Patients Receiving Best Supportive Care

In patients treated with best supportive care only in the NCIC CTG/AGITG CO.17 trial (n=195), those with p.G13D-mutated tumors (n=13) had significantly worse overall survival (median, 3.6 [95% CI, 2.2-4.8] months) than those with other *KRAS*-mutated tumors (median, 4.7 [95% CI, 3.6-6.7] months; HR, 1.90; 95% CI, 1.03-3.51; $P=.04$) or wild-type *KRAS* tumors (median, 5.0 [95% CI, 4.2-5.5] months; HR, 1.90; 95% CI, 1.05-3.41; $P=.03$) in univariate analysis (TABLE 2). In the multivariate analysis adjusting for potential prognostic factors, this difference became nonsignificant for the comparison between patients with p.G13D-mutated and other *KRAS*-mutated tumors (adjusted HR, 1.39; 95% CI, 0.73-2.64; $P=.33$) and between p.G13D-mutated and *KRAS* wild-type tumors (adjusted HR, 1.82; 95% CI, 0.99-3.34; $P=.053$). No significant difference in progression-free survival was seen for the 3 *KRAS* groups (Table 2). No analysis was performed for response rate

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because no patient responded under best supportive care.

Association of p.G13D Mutation With Outcome in Patients Receiving Cetuximab

Among patients who received any cetuximab-based treatment (cetuximab monotherapy or cetuximab plus chemotherapy) (n=571), overall and progression-free survival were significantly longer in patients with p.G13D-mutated tumors (overall survival: n=32; median, 7.6 [95% CI, 5.7-20.5] months; progression-free survival, n=32; median, 4.0 [95% CI, 1.9-6.2] months) than in patients with other *KRAS*-mutated tumors (overall survival: median, 5.7 [95% CI, 4.9-6.8] months; progression-free survival: median, 1.9 [95% CI, 1.8-2.8] months) in both univariate analysis (overall survival: HR, 0.52; 95% CI, 0.33-0.80; *P*=.003; progression-free survival: HR, 0.54; 95% CI, 0.36-0.81; *P*=.02) and multivariate analysis adjusting for potential prognostic factors and data set (overall survival: HR, 0.50; 95% CI, 0.31-0.81; *P*=.005; progression-free survival: HR, 0.51; 95% CI, 0.32-0.81; *P*=.004) (TABLE 3). No significant difference in overall or progression-free survival was found between patients with p.G13D-mutated and *KRAS* wild-type tumors in either univariate analysis (*P*=.98 and *P*=.97, respectively) or multivariate analysis (*P*=.79 and *P*=.66, respectively) (Table 3). Response rate was not significantly different between patients with p.G13D-mutated and other *KRAS*-mutated tumors (2/32 [6.3%; 95% CI, 0%-14.6%] vs 3/188 [1.6%; 95% CI, 0%-3.3%], respectively; *P*=.15), but patients with *KRAS* wild-type tumors had a significantly higher response rate than patients with p.G13D-mutated tumors (91/345 [26.4%; 95% CI, 21.7%-31.0%] vs 2/32 [6.3%; 95% CI, 0%-14.6%], respectively; *P*=.02) (TABLE 4).

Subgroup analyses of outcome according to treatment (cetuximab monotherapy or cetuximab plus chemotherapy) are presented in Table 3. In the cetuximab plus chemotherapy subgroup, a statistically significant association between the p.G13D mutation and better outcome after cetuximab treatment was observed, unlike in patients with other *KRAS*-mutated tumors. Patients with p.G13D-mutated tumors (n=22), compared with those with other *KRAS*-mutated tumors, had significantly longer overall survival (median, 10.6 [95% CI, 5.7-24.6] months vs 7.4 [95% CI, 5.5-9.0] months; adjusted HR, 0.46; 95% CI, 0.24-0.86; *P*=.02), longer progression-free survival (median, 4.1 [95% CI, 2.8-6.9] months vs 2.8 [95% CI, 2.5-3.7] months; adjusted HR, 0.49; 95% CI, 0.28-0.86; *P*=.01) (Table 3), and higher response rate (2/22 [9.1%; 95% CI, 0%-21.1%] vs 1/99 [1.0%; 95% CI, 0%-3.0%]; *P*=.08). No significant difference in either overall or progression-free survival was found between patients

Table 3. Overall and Progression-Free Survival in Cetuximab-Treated Patients

Survival	*KRAS* Mutation: *KRAS* p.G13D Mutation (n = 45)	*KRAS* Mutation: Other *KRAS* Mutation (n = 265)	*KRAS* Wild-Type (n = 464)
Overall survival			
Any cetuximab-based treatment, No.	32	195	345
Median survival (95% CI), mo	7.6 (5.7-20.5)	5.7 (4.9-6.8)	10.1 (9.4-11.3)
Univariate HR (95% CI)[a]	1 [Reference]	0.52 (0.33-0.80)	1.01 (0.66-1.54)
Log-rank *P* value		.003	.98
Multivariate HR (95% CI)[a]	1 [Reference]	0.50 (0.31-0.81)	0.94 (0.60-1.48)
Cox regression *P* value		.005	.79
Cetuximab monotherapy, No.	10	91	146
Median survival (95% CI), mo	6.7 (3.3-20.5)	4.8 (4.0-5.9)	9.4 (7.7-10.3)
Univariate HR (95% CI)[a]	1 [Reference]	0.66 (0.32-1.38)	0.86 (0.41-1.78)
Log-rank *P* value		.27	.68
Multivariate HR (95% CI)[a]	1 [Reference]	0.74 (0.34-1.64)	0.70 (0.31-1.62)
Cox regression *P* value		.46	.41
Cetuximab plus chemotherapy, No.	22	104	199
Median survival (95% CI), mo	10.6 (5.7-24.6)	7.4 (5.5-9.0)	11.3 (9.9-13.6)
Univariate HR (95% CI)[a]	1 [Reference]	0.50 (0.29-0.88)	1.06 (0.62-1.81)
Log-rank *P* value		.01	.96
Multivariate HR (95% CI)[a]	1 [Reference]	0.46 (0.24-0.86)	1.0 (0.56-1.79)
Cox regression *P* value		.02	>.99
Progression-free survival			
Any cetuximab-based treatment, No.	32	194	347
Median survival (95% CI), mo	4.0 (1.9-6.2)	1.9 (1.8-2.8)	4.2 (3.9-5.4)
Univariate HR (95% CI)[a]	1 [Reference]	0.54 (0.36-0.81)	0.99 (0.68-1.45)
Log-rank *P* value		.02	.97
Multivariate HR (95% CI)[a]	1 [Reference]	0.51 (0.32-0.81)	1.10 (0.72-1.69)
Cox regression *P* value		.004	.66
Cetuximab monotherapy, No.	10	91	146
Median survival (95% CI), mo	1.8 (1.7-11.0)	1.8 (1.8-1.9)	3.7 (2.8-4.1)
Univariate HR (95% CI)[a]	1 [Reference]	0.49 (0.23-1.03)	0.72 (0.35-1.48)
Log-rank *P* value		.05	.37
Multivariate HR (95% CI)[a]	1 [Reference]	0.60 (0.25-1.40)	0.63 (0.27-1.49)
Cox regression *P* value		.24	.29
Cetuximab plus chemotherapy, No.	22	103	201
Median survival (95% CI), mo	4.1 (2.8-6.9)	2.8 (2.5-3.7)	5.5 (4.2-5.5)
Univariate HR (95% CI)[a]	1 [Reference]	0.63 (0.39-1.03)	1.23 (0.79-1.94)
Log-rank *P* value		.06	.44
Multivariate HR (95% CI)[a]	1 [Reference]	0.49 (0.28-0.86)	1.30 (0.78-2.16)
Cox regression *P* value		.01	.31

Abbreviations: CI, confidence interval; HR, hazard ratio.
[a]Hazard ratios are expressed for comparison of *KRAS* p.G13D mutation vs other status.

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Table 4. Response Rates in Cetuximab-Treated Patients

Objective Response	*KRAS* Mutation		*KRAS* Wild-Type (n = 464)
	KRAS p.G13D Mutation (n = 45)	Other *KRAS* Mutation (n = 265)	
Any cetuximab-based treatment, No.	32	188	345
Response rate, No. (%) [95% CI]	2/32 (6.3) [0-14.6]	3/188 (1.6) [0-3.3]	91/345 (26.4) [21.7-31.0]
Univariate OR (95% CI)[a]	1 [Reference]	4.28 (0.69-26.70)	0.19 (0.05-0.82)
Fisher exact test *P* value		.15	.02
Multivariate OR (95% CI)[a]	1 [Reference]	3.64 (0.53-25.13)	0.16 (0.04-0.72)
Logistic regression *P* value		.19	.02
Cetuximab monotherapy, No.	10	89	146
Response rate, No.(%) [95% CI]	0/10 (0)	2/89 (2.3) [0-5.3]	23/146 (15.8) [9.8-21.7]
Univariate OR (95% CI)[a]	1 [Reference]	NC	NC
Fisher exact test *P* value		.94	.36
Multivariate OR (95% CI)[a]	1 [Reference]	NC	NC
Logistic regression *P* value		.97	.97
Cetuximab + chemotherapy, No.	22	99	199
Response rate No.(%) [95% CI]	2/22 (9.1) [0-21.1]	1/99 (1.0) [0-3.0]	68/199 (34.2) [27.6-40.8]
Univariate OR (95% CI)[a]	1 [Reference]	10.42 (0.90-120.8)	0.20 (0.05-0.90)
Fisher exact test *P* value		.08	.03
Multivariate OR (95% CI)[a]	1 [Reference]	8.63 (0.71-105.6)	0.22 (0.05-0.97)
Logistic regression *P* value		.09	.046

Abbreviations: CI, confidence interval; NC, not computed; OR, odds ratio.
[a]Odds ratios are expressed for comparison of *KRAS* p.G13D mutation vs other status.

with p.G13D-mutated and *KRAS* wild-type tumors in either of these 2 subgroups ($P = .41$ and $P > .99$ for overall survival and $P = .29$ and $P = .31$ for progression-free survival in the cetuximab monotherapy and cetuximab plus chemotherapy groups, respectively) (Table 3).

However, patients with *KRAS* wild-type tumors receiving cetuximab with chemotherapy had a significantly higher response rate than patients with p.G13D-mutated tumors (68/199 [34.2%; 95% CI, 27.6%-40.8%] vs 2/22 [9.1%; 95% CI, 0%-21.1%], respectively; $P = .03$). FIGURE 1 and FIGURE 2 show predictive effects by *KRAS* status. Among patients who received any cetuximab-based treatment, those with p.G13D-mutated tumors had a longer median overall survival (7.6 [95% CI, 5.7-20.5] months) and progression-free survival (4.0 [95% CI, 1.9-6.2] months) than those receiving best supportive care alone (3.6 [95% CI, 2.2-4.8] months and 1.7 [95% CI, 1.5-1.7] months, respectively), which were significant in univariate analysis (overall survival: HR, 0.24; 95% CI, 0.12-0.51; $P < .001$; progression-free survival: HR, 0.39; 95% CI, 0.20-0.78; $P = .006$) but became nonsignificant in multivariate analysis (overall survival: adjusted HR, 0.40; 95% CI, 0.13-1.28; $P = .12$; progression-free survival: HR, 0.53; 95% CI, 0.16-1.73; $P = .29$).

For patients with other *KRAS* mutations, no significant difference in overall survival was found between any and no cetuximab treatment (median, 5.7 [95% CI, 4.9-6.8] months vs 4.7 [95% CI, 3.6-6.7] months; adjusted HR, 1.07; 95% CI, 0.74-1.60; $P = .71$), while a significant difference was found in progression-free survival (median, 1.9 [95% CI, 1.8-2.8] months vs 1.8 [95% CI, 1.7-1.9] months) in univariate analysis (HR, 0.69; 95% CI, 0.52-0.92; $P = .01$) but not in multivariate analysis (adjusted HR, 0.93; 95% CI, 0.71-1.39; $P = .96$). As expected, patients with *KRAS* wild-type tumors receiving any cetuximab-based treatment, compared with patients receiving best supportive care alone, had significantly longer median overall survival (10.1 [95% CI, 9.4-11.3] months vs 5.0 [95% CI, 4.2-5.5] months, respectively; adjusted HR, 0.60; 95% CI, 0.44-0.81; $P < .001$) and progression-free survival (4.2 [95% CI, 3.9-5.4] months vs 1.9 [95% CI, 1.8-2.0] months; adjusted HR, 0.42; 95% CI, 0.32-0.56; $P < .001$).

We performed a test for interaction between any vs no cetuximab treatment and p.G13D vs other *KRAS* mutations. The adjusted *P* value for the interaction was $P = .003$ (HR, 0.30; 95% CI, 0.14-0.67) for overall survival and $P = .05$ (HR, 0.47; 95% CI, 0.22-1.00) for progression-free survival. There was no interaction between p.G13D mutation vs wild-type *KRAS* status and overall survival benefit from cetuximab-based treatment (any vs none) (HR, 0.49; 95% CI, 0.23-1.04; $P = .06$).

In univariate analysis, patients with p.G13D-mutated tumors had a significantly longer overall survival compared with patients receiving best supportive care (any cetuximab: HR, 0.24; 95% CI, 0.11-0.50; $P < .001$; cetuximab monotherapy: HR, 0.34; 95% CI, 0.13-0.87; $P = .02$). Similarly, progression-free survival was superior in p.G13D patients receiving any cetuximab (HR, 0.39; 95% CI, 0.19-0.78; $P = .006$) and cetuximab monotherapy (HR, 0.38; 95% CI, 0.15-0.98; $P = .04$) compared with patients receiving best supportive care alone. Although the differences were no longer statistically significant in multivariate analysis, the significant *P* value for interaction confirms that in this data set, the p.G13D mutation was associated with significantly greater overall survival benefit than tumors expressing other *KRAS* mutations (Figure 2 and FIGURE 3).

For patients with other *KRAS*-mutated tumors, progression-free survival was significantly longer when receiving cetuximab with chemotherapy than when receiving best supportive care alone (median, 2.8 [95% CI, 2.5-3.7] months vs 1.8 [95% CI, 1.7-1.9] months; adjusted HR, 0.53; 95% CI, 0.36-0.79; $P = .002$) but not when re-

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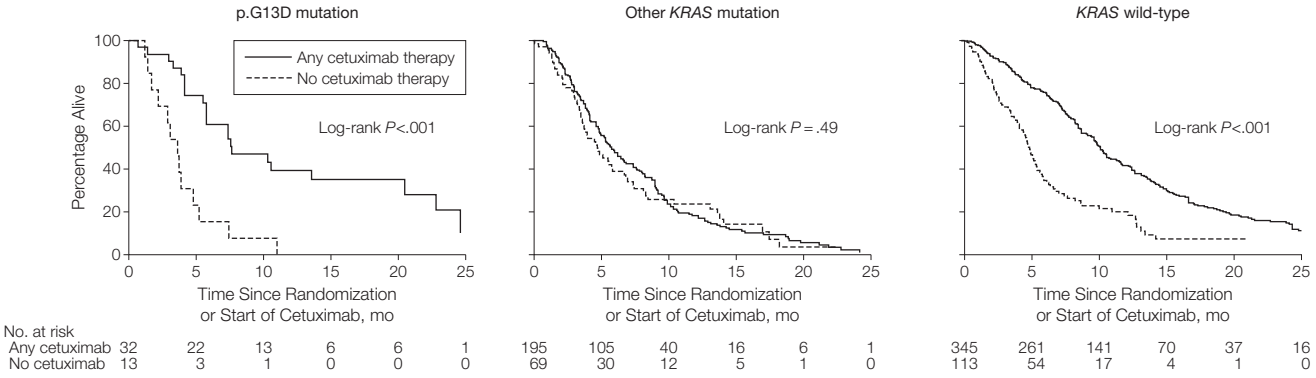
ceiving cetuximab as monotherapy (Figure 2), suggesting some retained chemosensitivity in these patients. Regardless of whether cetuximab was received with or without chemotherapy, patients with *KRAS* wild-type tumors, compared with those receiving best supportive care alone, had significantly longer median survival (cetuximab plus chemotherapy: median, 11.3 [95% CI, 9.9-13.6] months vs 5.0 [95% CI, 4.2-5.5] months; adjusted HR, 0.44; 95% CI, 0.32-0.62; $P < .001$; cetuximab monotherapy: median, 9.4 [95% CI, 7.7-10.3] months vs 5.0 [95% CI, 4.2-5.5] months; adjusted HR, 0.58; 95% CI, 0.42-0.78; $P < .001$) and progression-free survival (cetuximab plus chemotherapy: median, 5.5 [95% CI, 4.2-5.5] months vs 1.9 [95% CI, 1.8-2.0] months; adjusted HR, 0.22; 95%

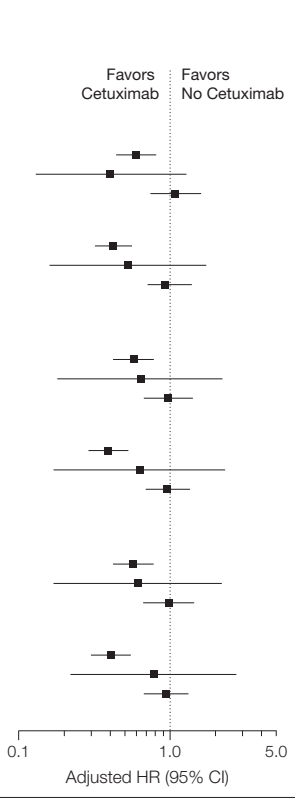
Figure 1. Overall Survival: Predictive Analysis by *KRAS* Status for Patients Receiving Any Cetuximab-Based Therapy vs No Cetuximab



The no cetuximab group for all patients from the pooled data set is the best supportive care group from the CO.17 trial.

Figure 2. Forest Plot of Hazard Ratios (HRs) for Overall and Progression-Free Survival With and Without Cetuximab

KRAS Subset	Median Survival (95% CI), mo: Cetuximab	Median Survival (95% CI), mo: No Cetuximab	Adjusted HR (95% CI)	*P* for Interaction: p.G13D vs *KRAS* Wild-Type	*P* for Interaction: p.G13D vs Other *KRAS* Mutation
Any cetuximab vs no cetuximab					
Overall survival					
KRAS wild-type	10.1 (9.4-11.3)	5.0 (4.2-5.5)	0.60 (0.44-0.81)	.06	.003
p.G13D mutation	7.6 (5.7-20.5)	3.6 (2.2-4.8)	0.40 (0.13-1.28)		
Other *KRAS* mutation	5.7 (4.9-6.8)	4.7 (3.6-6.7)	1.07 (0.74-1.60)		
Progression-free survival					
KRAS wild-type	4.2 (3.9-5.4)	1.9 (1.8-2.0)	0.42 (0.32-0.56)	.79	.05
p.G13D mutation	4.0 (1.9-6.2)	1.7 (1.5-1.7)	0.53 (0.16-1.73)		
Other *KRAS* mutation	1.9 (1.8-2.8)	1.8 (1.7-1.9)	0.93 (0.71-1.39)		
Cetuximab monotherapy vs no cetuximab					
Overall survival					
KRAS wild-type	9.4 (7.7-10.3)	5.0 (4.2-5.5)	0.58 (0.42-0.78)	.15	.13
p.G13D mutation	6.7 (3.3-20.5)	3.6 (2.2-4.8)	0.64 (0.18-2.21)		
Other *KRAS* mutation	4.8 (4.0-5.9)	4.7 (3.6-6.7)	0.97 (0.67-1.42)		
Progression-free survival					
KRAS wild-type	3.7 (2.8-4.1)	1.9 (1.8-2.0)	0.39 (0.29-0.53)	.36	.07
p.G13D mutation	1.8 (1.7-11.0)	1.7 (1.5-1.7)	0.63 (0.17-2.30)		
Other *KRAS* mutation	1.8 (1.8-1.9)	1.8 (1.7-1.9)	0.96 (0.69-1.35)		
Cetuximab monotherapy vs no cetuximab in CO.17 trial only					
Overall survival					
KRAS wild-type	9.4 (7.7-10.3)	5.0 (4.2-5.5)	0.58 (0.42-0.78)	.22	.15
p.G13D mutation	5.5 (3.0-NA)	3.6 (2.2-4.8)	0.61 (0.17-2.19)		
Other *KRAS* mutation	4.7 (3.8-5.6)	4.7 (3.6-6.7)	0.99 (0.67-1.44)		
Progression-free survival					
KRAS wild-type	3.7 (3.1-5.1)	1.9 (1.8-2.0)	0.41 (0.30-0.55)	.92	.33
p.G13D mutation	1.7 (1.6-1.9)	1.7 (1.5-1.7)	0.78 (0.22-2.74)		
Other *KRAS* mutation	1.8 (1.7-1.8)	1.8 (1.7-1.9)	0.96 (0.68-1.34)		



Comparisons include any cetuximab therapy (with or without chemotherapy) vs no cetuximab, cetuximab monotherapy vs no cetuximab, and a sensitivity analysis including only those randomized from the CO.17 trial (cetuximab monotherapy vs no cetuximab). *P* values for interaction (adjusted for predefined prognostic factors) indicate capacity of biomarker to differentiate outcomes between *KRAS* mutation status subgroups. CI indicates confidence interval; NA, not enough data to estimate.

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CI, 0.16-0.31; $P<.001$; cetuximab monotherapy: median, 3.7 [95% CI, 2.8-4.1] months vs 1.9 [95% CI, 1.8-2.0] months; adjusted HR, 0.39; 95% CI, 0.29-0.53; $P<.001$) (Figure 2).

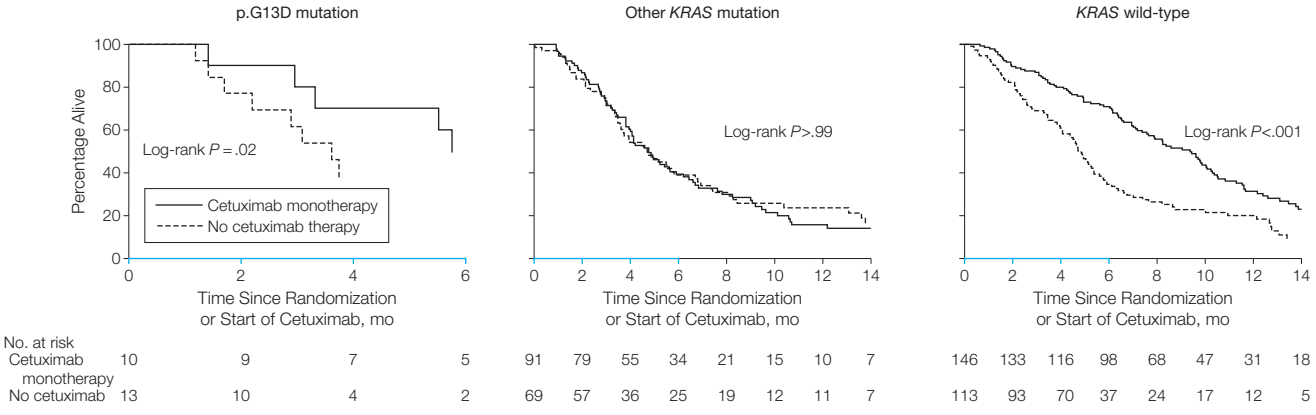
A separate analysis of the CO.17 trial (n=195), containing the only randomized patients (cetuximab plus best supportive care vs best supportive care alone) in the pooled data set, was performed as a sensitivity analysis to avoid the potential bias associated with cross-trial comparisons and to allow the purest assessment of the impact of *KRAS* mutations on the effect of cetuximab.

Within the p.G13D mutation subset (n=13), the adjusted HR for overall survival for cetuximab therapy compared with best supportive care alone was in the same direction as in the cross-trial comparison, although the *P* value was not significant (adjusted HR, 0.61; 95% CI, 0.17-2.19; $P=.45$). There was no benefit for cetuximab therapy in the other *KRAS* mutations subset (adjusted HR, 0.99; 95% CI, 0.67-1.44; $P=.94$). There was a significant interaction between p.G13D mutation status (p.G13D vs other *KRAS* mutations) and overall survival benefit from therapy (cetuximab vs best supportive care) in the univariate analysis (HR, 0.33; 95% CI, 0.11-1.00; $P=.05$), which was not significant in the multivariate analysis (adjusted HR, 0.43; 95% CI, 0.14-1.34; $P=.15$) (FIGURE 4).

In Vitro and In Vivo Effects of p.G13D Mutation on Cetuximab Sensitivity

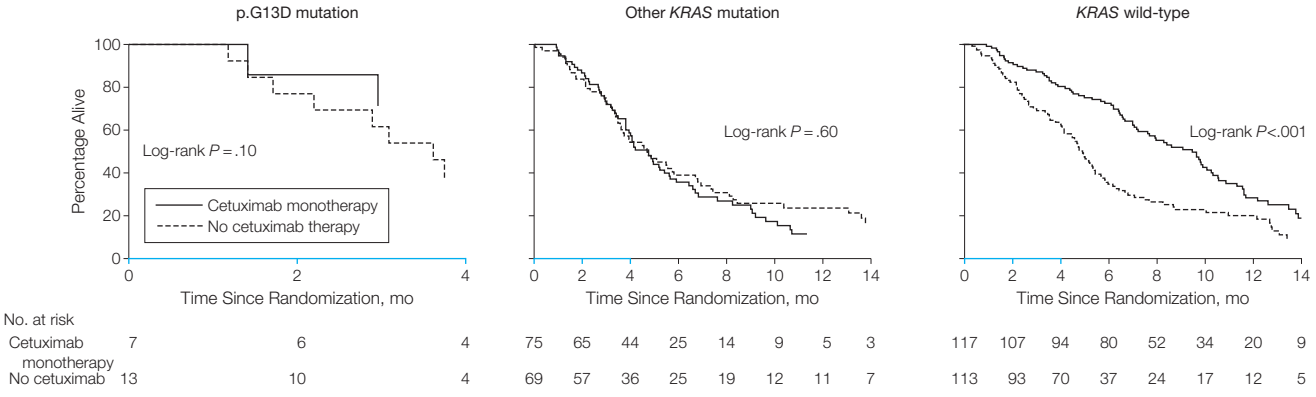
We introduced p.G12V and p.G13D alleles in the genome of human colorectal SW48 cells by targeted homologous recombination (eAppendix).[23] While the proliferation of p.G12V-mutated SW48 cells was unaffected by cetuximab, the isogenic p.G13D-mutated cells displayed a drug response similar to their wild-type counterpart (eFigure 1, A). Importantly, the proliferative capabilities of parental and *KRAS*-mutated cells were undistinguishable (eFigure 1, B). Cetuximab administration prominently inhibited the growth of tumors formed by wild-type or *KRAS* p.G13D mutant cells

Figure 3. Overall Survival: Predictive Analysis by *KRAS* Status for Patients Receiving Cetuximab Monotherapy vs No Cetuximab



The no cetuximab group for all patients from the pooled data set is the best supportive care group from the CO.17 trial. Horizontal axis shown in blue indicates range of time since randomization from 0 through 6 months.

Figure 4. Overall Survival: Predictive Analysis by *KRAS* Status for Patients Receiving Cetuximab Monotherapy vs No Cetuximab in the CO.17 Trial Only



Horizontal axis shown in blue indicates range of time since randomization from 0 through 4 months.

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grown as xenografts in immunocompromised mice (eFigure 1, C). In contrast, the growth of tumors formed by the *KRAS* p.G12V cells was not significantly affected by cetuximab treatment (eFigure 1, C). We then measured the level of activation (phosphorylation status) of EGFR and its downstream effectors (mitogen-activated protein kinase/extracellular signal-regulated kinase and v-akt murine thymoma viral oncogene homolog) in *KRAS* wild-type and mutant SW48 cells. In the presence of cetuximab, the p.G12V-mutated cells seemingly could still activate the extracellular signal-regulated kinase pathway but the p.G13D-mutated cells could not (eFigure 2). Of note, the levels of activated *KRAS* (guanosine-5′-triphosphate-bound) were similar in p.G12V- and p.G13D-mutated cells (eFigure 3). Overall, these results indicate that the *KRAS* p.G12V and p.G13D mutations differently affect response to cetuximab in preclinical models.

COMMENT

In a large, retrospective pooled exploratory analysis of patients with chemotherapy-refractory colorectal cancer, we show for the first time that there is a positive association between *KRAS* p.G13D mutations and cetuximab treatment in regard to better overall and progression-free survival.

The improved survival observed in patients with p.G13D-mutated tumors in the cetuximab monotherapy group suggests that p.G13D-mutated tumors may be sensitive to cetuximab and precludes a chemotherapy-driven effect. Patients with p.G13D-mutated tumors treated with combination regimens also have significantly better overall survival than do those with other *KRAS*-mutated tumors, which mirrors the observation in monotherapy-treated patients, suggesting cetuximab-dependent effects, although it cannot be excluded that chemotherapy is a confounding factor in patients treated with cetuximab plus chemotherapy.

In the monotherapy group, the difference in overall and progression-free survival between patients with p.G13D-mutated tumors and those with other *KRAS*-mutated tumors was not statistically significant. However, the magnitude of the effect was comparable and the direction was the same as in the patients treated with cetuximab plus chemotherapy.

Because this is a pooled analysis, to reduce the risk of biases implicit in this kind of study, we adjusted for type of previous treatment (whether all 3 chemotherapy drugs [fluoropyrimidine, irinotecan, and oxaliplatin] were previously received) and data set in the multivariate analyses. However, in the absence of randomization, there may be inadequate controlling for unknown confounders. For the predictive analysis and the estimation of treatment effect of cetuximab over no cetuximab, the comparator group for all patients from the pooled data set was the best supportive care group from the CO.17 trial. The sensitivity analysis from the CO.17 trial provided an unbiased estimate that was consistent with the finding of the pooled analysis, although it was not statistically significant.

To study the association of the p.G13D mutation with outcome in metastatic colorectal cancer, we compared overall and progression-free survival between the different *KRAS* mutation groups in the 195 patients in the CO.17 trial randomized to best supportive care alone. In this subset, the 13 patients with p.G13D-mutated tumors had a worse overall survival than those with *KRAS* wild-type tumors and those with tumors bearing other *KRAS* mutations, in univariate but not in multivariate analysis. Of particular relevance, patients with p.G13D-mutated tumors in our series also seemed to benefit more from cetuximab treatment than those with *KRAS* wild-type tumors, suggesting that the poor prognosis of a p.G13D mutation is mitigated by cetuximab treatment. Given the relatively small number of patients with p.G13D-mutated tumors, caution in drawing conclusions is warranted. Comparing overall survival between patients with p.G13D-mutated vs other *KRAS*-mutated and *KRAS* wild-type colorectal cancers in the control groups of randomized trials will contribute to determining whether this is a true association.

Although p.G13D-mutated tumors do not behave like other *KRAS*-mutated tumors, they appear to behave somewhat differently than *KRAS* wild-type tumors. Our results indicate that patients with p.G13D-mutated tumors respond to cetuximab therapy, albeit with a lower response rate than those with *KRAS* wild-type tumors.

The prolonged progression-free and overall survival of patients with p.G13D-mutated tumors in comparison with those with other *KRAS*-mutated tumors may not be due to a real reduction in tumor burden but to a delay in progression. A possible explanation of this clinical observation is that p.G13D mutant tumors do not undergo apoptosis (cytotoxic effect) on EGFR inhibition, but proliferation is inhibited (cytostatic effect).

When assessing the effect of cetuximab treatment on cellular proliferation in SW48 isogenic clones carrying p.G12V or p.G13D mutations, we found that while p.G12V-mutated cells were insensitive to cetuximab, p.G13-mutated cells were nearly as responsive to cetuximab as wild-type cells. These results provide a cell-based molecular explanation to our clinical observation that patients with p.G13D-mutated tumors benefit from cetuximab treatment, while those with other *KRAS*-mutated tumors do not.

Our study is limited because it is a retrospective observational study that relies largely on nonrandomized or cross-trial comparisons for discussion of possible treatment effects. It can therefore only suggest an association between p.G13D mutation status and survival benefit after cetuximab-based treatment. The results from the patient sample analyses are supported by isogenic cellular models, wherein the different effects of the G12 and G13 *KRAS* alleles on response to cetuximab are evident.

In conclusion, our study retrospectively observed an association be-

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tween the presence of a p.G13D mutation and survival benefit in chemotherapy-refractory metastatic colorectal cancer treated with cetuximab. Prospective randomized trials are needed before conclusions about potential beneficial effects of cetuximab in p.G13D-mutated chemotherapy-refractory metastatic colorectal cancer should be inferred.

Author Affiliations: Center for Human Genetics, University of Leuven, Leuven, Belgium (Drs De Roock and Tejpar); Ottawa Hospital Research Institute, University of Ottawa, Ottawa, Ontario, Canada (Dr Jonker); Laboratory of Molecular Genetics, Institute for Cancer Research and Treatment, University of Turin Medical School, Turin, Italy (Drs Di Nicolantonio, Arena, and Bardelli and Ms Lamba); Italian Foundation for Cancer Research, Institute of Molecular Oncology (Drs Di Nicolantonio and Bardelli), and the Falck Division of Medical Oncology, Ospedale Niguarda Ca'Granda (Drs Sartore-Bianchi and Siena), Milano, Italy; National Cancer Institute of Canada Clinical Trials Group, Kingston, Ontario, Canada (Drs Tu and O'Callaghan); Laboratory of Molecular Diagnostics, Institute of Pathology, Locarno, Switzerland (Dr Frattini); Cliniques Universitaires Saint-Luc, Brussels, Belgium (Dr Piessevaux); Digestive Oncology Unit, University Hospital Gasthuisberg, Leuven, Belgium (Drs Van Cutsem and Tejpar); Bristol-Myers Squibb Research and Development, Princeton, New Jersey (Dr Khambata-Ford); Peter MacCallum Cancer Centre, University of Melbourne, and Australasian Gastrointestinal Trials Group, Melbourne, Australia (Dr Zalcberg); National Health and Medical Research Council Clinical Trials Centre, University of Sydney, Sydney, Australia (Dr Simes); and Flinders Medical Centre and Flinders University, Adelaide, Australia (Dr Karapetis). Dr De Roock is now with the Department of General Medical Oncology, University Hospital Gasthuisberg.

Author Contributions: Dr Tejpar had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Drs De Roock, Jonker, and Di Nicolantonio contributed equally to this article. Drs De Roock and Jonker wrote the first draft of the manuscript and were equally involved in study concept and design, clinical data collection, and clinical data analysis. Dr Di Nicolantonio was primarily responsible for all in vitro and in vivo data.

Study concept and design: De Roock, Jonker, Di Nicolantonio, Siena, Bardelli, Tejpar.

Acquisition of data: De Roock, Jonker, Di Nicolantonio, Sartore-Bianchi, Tu, Siena, Lamba, Arena, Van Cutsem, O'Callaghan, Khambata-Ford, Simes, Tejpar.

Analysis and interpretation of data: De Roock, Jonker, Di Nicolantonio, Sartore-Bianchi, Tu, Siena, Lamba, Arena, Frattini, Piessevaux, O'Callaghan, Khambata-Ford, Zalcberg, Simes, Karapetis, Bardelli, Tejpar.

Drafting of the manuscript: De Roock, Jonker, Bardelli, Tejpar.

Critical revision of the manuscript for important intellectual content: De Roock, Jonker, Di Nicolantonio, Sartore-Bianchi, Tu, Siena, Lamba, Arena, Frattini, Piessevaux, Van Cutsem, O'Callaghan, Khambata-Ford, Zalcberg, Simes, Karapetis, Bardelli, Tejpar.

Statistical analysis: De Roock, Jonker, Di Nicolantonio, Tu, Piessevaux, Karapetis, Tejpar.

Obtained funding: Simes, Bardelli, Tejpar.

Administrative, technical, or material support: Khambata-Ford, Tejpar.

Study supervision: Jonker, Bardelli, Tejpar.

Financial Disclosures: Dr Siena reports serving as a member of advisory boards for Merck Serono, Amgen, AstraZeneca, and Celgene. Dr Van Cutsem reports receiving research funding from Merck Serono and Amgen. Dr Khambata-Ford reports owning equity in and is an employee of Bristol-Myers Squibb. Dr Zalcberg reports receiving travel and research support from Merck Serono and research support from Bristol-Myers Squibb. Dr Simes reports that his institution has received research grants from Bristol-Myers Squibb. Dr Karapetis reports serving as a member of an advisory board for Merck Serono. Dr Bardelli reports that he owns stock in and has received consultancy fees from Horizon Discovery. Dr Tejpar reports receiving research grants from Merck Serono. No other disclosures were reported.

Funding/Support: Dr De Roock is supported by a PhD grant from the Institute for the Promotion of Innovation Through Science and Technology in Flanders (IWT-Vlaanderen). Drs Sartore-Bianchi and Siena are supported by Associazione Italiana Ricerca Cancro and Oncologia Ca'Granda Onlus Fondazione. Drs Van Cutsem and Tejpar are senior clinical investigators for the Fund for Scientific Research, Flanders and received research grants from the Belgian Federation Against Cancer.

Role of the Sponsor: The funding sources had no role in design and conduct of the study; collection, management, analysis, and interpretation of the data; or preparation, review, or approval of the manuscript.

Online-Only Material: The eAppendix, the eTable, and eFigures 1 through 3 are available online at http://www.jama.com.

Additional Contributions: We acknowledge Jef De Schutter, MSc, and Bart Biesmans, MSc, Digestive Oncology Unit, University Hospital Gasthuisberg, for laboratory analysis support, and Nancy Gustafson, MS, Daniel Malone, BS, and Christopher T. Harbison, PhD, Bristol-Myers Squibb, for laboratory and statistical analysis support. They did not receive compensation for their work.

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