JOURNAL OF CLINICAL ONCOLOGY

ORIGINAL REPORT

Cetuximab Plus Irinotecan, Fluorouracil, and Leucovorin As First-Line Treatment for Metastatic Colorectal Cancer: Updated Analysis of Overall Survival According to Tumor *KRAS* and *BRAF* Mutation Status

Eric Van Cutsem, Claus-Henning Köhne, István Láng, Gunnar Folprecht, Marek P. Nowacki, Stefano Cascinu, Igor Shchepotin, Joan Maurel, David Cunningham, Sabine Tejpar, Michael Schlichting, Angela Zubel, Ilhan Celik, Philippe Rougier, and Fortunato Ciardiello

From the University Hospital Gasthuisberg, Leuven, Belgium; Klinikum Oldenburg, Oldenburg; University Hospital Carl Gustav Carus, Dresden; Merck KGaA, Darmstadt, Germany; National Institute of Oncology, Budapest, Hungary; MSC Memorial Cancer Center, Warsaw, Poland; Università Politecnica delle Marche, Ancona; Second University of Naples, Naples, Italy; National Cancer Institute, Kiev, Ukraine; Hospital Clínic, Barcelona, Spain; The Royal Marsden National Health Services Foundation Trust, London, United Kingdom; and the University of Versailles St Quentin, Hôpital Européen Georges Pompidou, Paris, France.

Submitted October 30, 2010; accepted February 8, 2011; published online ahead of print at www.jco.org on April 18, 2011.

Supported by Merck KGaA, Darmstadt, Germany.

Presented in part at 45th Annual Meeting of the American Society of Clinical Oncology, Orlando, FL, May 29-June 2, 2009; the European Society for Medical Oncology's (ESMO's) 11th World Congress on Gastrointestinal Cancer, Barcelona, Spain, June 24-27, 2009; the joint European Cancer Organization's 15th and ESMO 34th Multidisciplinary World Congress, Berlin, Germany, September 20-24, 2009; the American Society of Clinical Oncology Gastrointestinal Cancers Symposium, Orlando, FL, January 22-24, 2010; the 46th Annual Meeting of the American Society of Clinical Oncology, Chicago, IL, June 4-8, 2010; and ESMO's 12th World Congress on Gastrointestinal Cancer, Barcelona, Spain, June 30-July 03, 2010.

Authors' disclosures of potential conflicts of interest and author contributions are found at the end of this article.

Clinical Trials repository link available on JCO.org.

Corresponding author: Eric Van Cutsem, MD, PhD, University Hospital Gasthuisberg, Herestraat 49, 3000 Leuven, Belgium; e-mail: eric.vancutsem@uz.kuleuven.ac.be.

0732-183X/11/2999-1/$20.00

DOI: 10.1200/JCO.2010.33.5091

ABSTRACT

Purpose
The addition of cetuximab to irinotecan, fluorouracil, and leucovorin (FOLFIRI) as first-line treatment for metastatic colorectal cancer (mCRC) was shown to reduce the risk of disease progression and increase the chance of response in patients with *KRAS* wild-type disease. An updated survival analysis, including additional patients analyzed for tumor mutation status, was undertaken.

Patients and Methods
Patients were randomly assigned to receive FOLFIRI with or without cetuximab. DNA was extracted from additional slide-mounted tumor samples previously used to assess epidermal growth factor receptor expression. Clinical outcome according to the tumor mutation status of *KRAS* and *BRAF* was assessed in the expanded patient series.

Results
The ascertainment rate of patients analyzed for tumor *KRAS* status was increased from 45% to 89%, with mutations detected in 37% of tumors. The addition of cetuximab to FOLFIRI in patients with *KRAS* wild-type disease resulted in significant improvements in overall survival (median, 23.5 *v* 20.0 months; hazard ratio [HR], 0.796; $P = .0093$), progression-free survival (median, 9.9 *v* 8.4 months; HR, 0.696; $P = .0012$), and response (rate 57.3% *v* 39.7%; odds ratio, 2.069; $P < .001$) compared with FOLFIRI alone. Significant interactions between *KRAS* status and treatment effect were noted for all key efficacy end points. *KRAS* mutation status was confirmed as a powerful predictive biomarker for the efficacy of cetuximab plus FOLFIRI. *BRAF* tumor mutation was a strong indicator of poor prognosis.

Conclusion
The addition of cetuximab to FOLFIRI as first-line therapy improves survival in patients with *KRAS* wild-type mCRC. *BRAF* tumor mutation is an indicator of poor prognosis.

J Clin Oncol 29. © 2011 by American Society of Clinical Oncology

INTRODUCTION

In pivotal phase III studies, the epidermal growth factor receptor (EGFR) –targeting monoclonal antibody, cetuximab, has been shown to improve the efficacy of standard chemotherapy regimens used in the first-line treatment of several common cancers, including metastatic colorectal cancer (mCRC).[1-3]

In particular, the CRYSTAL (Cetuximab Combined with Irinotecan in First-Line Therapy for Metastatic Colorectal Cancer) study met its primary end point in demonstrating that the addition of cetuximab to a combined first-line chemotherapy regimen of irinotecan, infusional fluorouracil, and leucovorin (FOLFIRI) statistically significantly reduced the risk of progression of metastatic colorectal cancer (mCRC) compared with chemotherapy alone (hazard ratio [HR], 0.85; $P = .048$).[1] The tumor response was also statistically significantly enhanced in the cetuximab plus FOLFIRI arm (odds ratio, 1.40; $P = .004$), as was the R0 resection rate of metastases with curative intent ($P = .002$). Overall survival, given a median time of follow-up of nearly 30 months, did not appear to be statistically significantly different between treatment groups (HR, 0.93; $P = .31$).[1]

Confirming earlier observations from single-arm studies,[4-7] and consistent with analyses in other randomized studies in mCRC involving cetuximab,[8,9] the clinical activity of cetuximab in the

CRYSTAL study was shown in a retrospective analysis to be limited to those patients whose tumors were wild-type at codons 12 and 13 of the *KRAS* gene, a group comprising 64% of the *KRAS* evaluable population. The benefit in patients with *KRAS* wild-type tumors was apparent in relation to a significantly reduced risk of disease progression (HR, 0.68; $P = .02$) and significantly increased odds of response in favor of the cetuximab plus FOLFIRI arm (odds ratio, 1.91). Overall survival also appeared to be improved in patients with *KRAS* wild-type tumors (HR, 0.84). No benefit for the addition of cetuximab to FOLFIRI was detected for any efficacy end point in patients whose tumors carried mutations of the *KRAS* gene (progression-free survival [PFS]: HR, 1.07; $P = .75$; overall survival: HR, 1.03; best overall response: odds ratio, 0.80). In the subgroup of patients evaluated for tumor *KRAS* mutation status, a significant interaction between treatment group and *KRAS* status was demonstrated for response ($P = .03$) but not (in this limited population) for PFS ($P = .07$) or overall survival ($P = .44$).

The *KRAS* analysis was based on the molecular typing of clinical material from a subgroup of 540 patients (45%) of the intention-to-treat (ITT; previously referred to as primary analysis[1]) population, (the *KRAS* population). Although comparison of the baseline characteristics and efficacy data suggested that the *KRAS* population was comparable to the ITT population, it was felt that a more accurate evaluation of the benefit of adding cetuximab to FOLFIRI as first-line treatment for mCRC would be obtained if tumor *KRAS* mutation status were to be determined for a higher proportion of patients. This article therefore reports an updated analysis of the CRYSTAL study, with increased follow-up time and

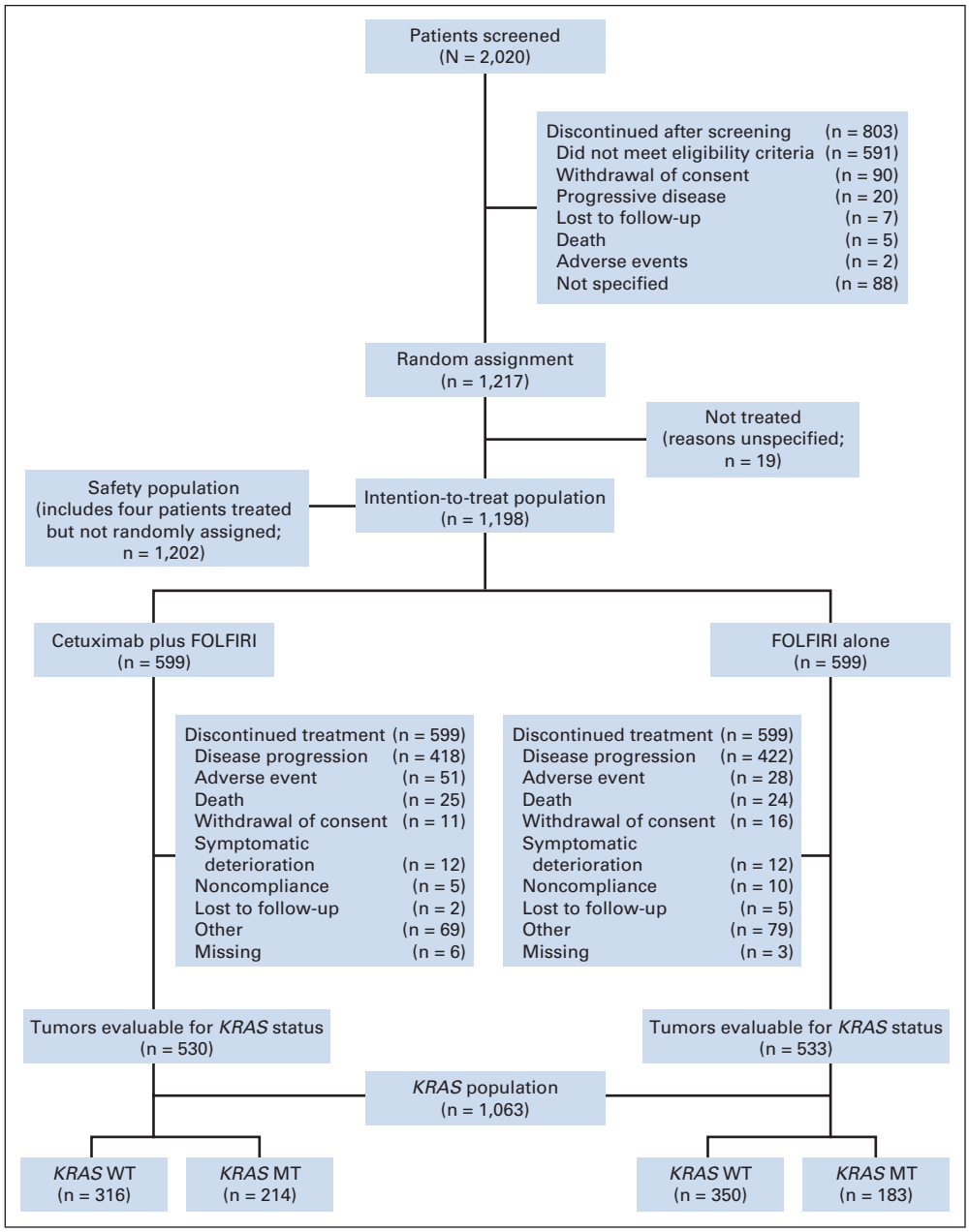

Fig 1. Disposition of patients and acquisition and analysis of clinical samples. FOLFIRI, irinotecan, leucovorin, and fluorouracil; WT, wild type; MT, mutant.

an increased number of patients evaluable for tumor *KRAS* status. The *BRAF* gene, which encodes a downstream effector of KRAS in the mitogen-activated protein kinase pathway,[10] is also mutated in a subset of mCRCs.[11-14] The clinical significance of the tumor mutation status of *BRAF* was considered in the expanded population of patients with *KRAS* wild-type tumors.

PATIENTS AND METHODS

Study Design and Treatment

Eligibility criteria and design have been described.[1] The study was carried out in accordance with the declaration of Helsinki (October 1996). All patients provided informed consent. This was an open-label, randomized, multicenter, phase III study comparing cetuximab plus FOLFIRI with FOLFIRI alone as first-line treatment for mCRC.

On day 1 of a 14-day treatment cycle, patients received cetuximab (initial dose 400 mg/m^2 infused over 2 hours, and 250 mg/m^2 weekly, over 1 hour, thereafter) followed after 1 hour by FOLFIRI (irinotecan 180 mg/m^2, day 1, infused over 30 to 90 minutes, followed by leucovorin 200 mg/m^2 L-form, or 400 mg/m^2 racemic, infused over 2 hours, followed by fluorouracil, as a 400 mg/m^2 intravenous bolus then a 2,400-mg/m^2 46-hour continuous infusion) or FOLFIRI alone, until disease progression or the occurrence of unacceptable toxicity.

Radiologic assessment of response was carried out every 8 weeks until disease progression or withdrawal. Toxicity was graded according to the National Cancer Institute Common Toxicity Criteria, version 2.0. Follow-up evaluations were performed every 3 months.

The primary end point was PFS, as determined by an independent review committee performing a preplanned, blinded review (based on modified WHO criteria) of radiological assessments. Secondary end points included overall survival, best overall response, and safety. A retrospective subgroup analysis investigated associations between tumor *KRAS* mutation status and outcome.

KRAS and BRAF Mutation Analysis

DNA was extracted from formalin-fixed paraffin-embedded (FFPE) tumor tissue and the mutation status of codons 12 and 13 of the *KRAS* gene assessed using a polymerase chain reaction clamping and melting curve technique, as previously described.[1] *BRAF* mutation status (V600E) was analyzed using a similar approach (LightMix *BRAF* V600E Kit; TIB MOLBIOL, Germany). The number of evaluable samples was increased from the previous analysis through the extraction of tumor DNA from slide-mounted tissue previously used to assess EGFR expression.

Statistical Methods and Considerations

The primary efficacy analysis of PFS was performed on the ITT population, which comprised all randomly assigned patients who received at least one dose of a study drug.[1] Prespecified analyses were repeated according to *KRAS* and *BRAF* tumor mutation status. PFS and overall survival times were analyzed by the Kaplan-Meier method[15] (product limit estimates) and stratified log-rank test. Best overall response rates were compared between treatment groups using Cochran-Mantel-Haenszel tests, stratified according to randomization strata. All reported *P* values were two sided and given the exploratory nature of the updated analyses, they have not been adjusted for multiple testing. HRs and odds ratios are expressed for cetuximab plus FOLFIRI versus FOLFIRI alone. The interaction of the treatment effect and tumor *KRAS* status was further explored for PFS and overall survival time using Cox models and

Table 1. Patient and Disease Characteristics at Baseline for ITT and *KRAS* Populations

Characteristic	CRYSTAL ITT Population (n = 1,198)		*KRAS* Population (n = 1,063)		*KRAS* Population			
					KRAS Wild-Type (n = 666)		*KRAS* Mutant (n = 397)	
	FOLFIRI (n = 599)	Cetuximab + FOLFIRI (n = 599)	FOLFIRI (n = 533)	Cetuximab + FOLFIRI (n = 530)	FOLFIRI (n = 350)	Cetuximab + FOLFIRI (n = 316)	FOLFIRI (n = 183)	Cetuximab + FOLFIRI (n = 214)
Sex, %								
Male	59.4	61.6	59.5	61.7	60.3	62.0	57.9	61.2
Female	40.6	38.4	40.5	38.3	39.7	38.0	42.1	38.8
Age, years								
Median	61	61	61	61	59	61	63	62
Range	19-84	22-82	19-84	22-80	19-84	24-79	32-83	22-80
Region, %								
Western Europe	44.6	43.7	45.4	44.9	45.1	45.9	45.9	43.5
Eastern Europe	33.6	33.9	35.5	36.0	35.1	36.4	36.1	35.5
Outside Europe	21.9	22.4	19.1	19.1	19.7	17.7	18.0	21.0
ECOG PS, %								
0	53.1	55.1	54.6	57.9	57.1	57.9	49.7	57.9
1	43.4	41.4	42.2	38.3	38.9	38.0	48.6	38.8
2	3.5	3.5	3.2	3.8	4.0	4.1	1.6	3.3
Laboratory values, %								
Lactate dehydrogenase > ULN	44.9	44.6	44.1	43.8	42.9	43.7	46.4	43.9
Alkaline phosphatase ≥ 300 U/L	13.2	11.9	12.2	11.3	12.0	9.5	12.6	14.0
Leukocyte count > 10,000/μL	19.9	15.7	19.7	15.3	16.6	15.2	25.7	15.4
Prior adjuvant chemotherapy, %	19.4	21.2	18.6	21.1	20.9	25.3	14.2	15.0
Metastases, %								
At one or two sites	83.5	86.1	84.1	86.8	84.3	87.7	83.6	85.5
Confined to liver	22.4	20.2	22.9	21.3	20.6	21.5	27.3	21.0

Abbreviations: ITT, intention-to-treat; CRYSTAL, Cetuximab Combined with Irinotecan in First-Line Therapy for Metastatic Colorectal Cancer; FOLFIRI, irinotecan, leucovorin and fluorouracil; ECOG PS, Eastern Cooperative Oncology Group performance status; ULN, upper limit of normal.

for best overall response rate using a logistic regression model by means of deviance tests. The impact of early acne-like rash (any grade) on outcome was explored using a landmark method.[16] Survival time was estimated conditionally according to whether the patient developed acne-like rash within 21 days (landmark period) after the start of study treatment (early acne-like rash). An evaluable population (patients under treatment at day 21) was determined which comprised all ITT patients alive on day 21 and with a treatment duration ≥ 21 days; survival times were calculated from the day of first dose of study medication.

Safety analyses were carried out on patients who received at least one dose of any study drug. Adverse events (AEs) were categorized according to the Medical Dictionary for Regulatory Activities (version 10.0) according to preferred terms and predefined special AE categories.

RESULTS

Patients and Samples

The ITT population comprised 1,198 randomly assigned and treated patients, with 599 receiving cetuximab plus FOLFIRI and 599 receiving FOLFIRI alone. The safety population (n = 1,202) included four additional patients who were treated but did not undergo random assignment, with 600 patients receiving cetuximab plus FOLFIRI and 602 FOLFIRI alone (Fig 1). Subsequent to the initial published analysis which had a cutoff date for overall survival of December 31, 2007, and an associated overall median duration of follow-up of 29.7 months, the extraction of DNA from tumor material recovered from FFPE slides used for immunohistochemical analysis of EGFR expression allowed for the typing of an additional 523 tumors for *KRAS* mutation status, representing an increase in the ascertainment rate from 45% of ITT population patients in the original analysis to 89% (540 to 1,063) in this analysis (*KRAS* population). An updated analysis of overall survival was therefore carried out with a new cutoff date of May 31, 2009, giving an overall median duration of follow-up of 46.8 months for patients receiving cetuximab plus FOLFIRI and 46.2 months for those receiving FOLFIRI alone.

Baseline characteristics were essentially well balanced between the treatment groups in the ITT and *KRAS* populations and between

Table 2. Efficacy Data for ITT and *KRAS* Populations

Parameter	CRYSTAL ITT Population (n = 1,198)		*KRAS* Population (n = 1063)		*KRAS* Population			
					KRAS Wild-Type (n = 666)		*KRAS* Mutant (n = 397)	
	FOLFIRI (n = 599)	Cetuximab + FOLFIRI (n = 599)	FOLFIRI (n = 533)	Cetuximab + FOLFIRI (n = 530)	FOLFIRI (n = 350)	Cetuximab + FOLFIRI (n = 316)	FOLFIRI (n = 183)	Cetuximab + FOLFIRI (n = 214)
Overall survival								
No. of events	502	487	444	430	288	242	156	188
Median, months	18.6	19.9	18.7	20.2	20.0	23.5	16.7	16.2
95% CI	16.7 to 19.8	18.5 to 21.3	16.8 to 20.3	18.7 to 21.8	17.4 to 21.7	21.2 to 26.3	14.9 to 19.4	14.9 to 17.9
Hazard ratio	0.878		0.888		0.796		1.035	
95% CI	0.774 to 0.995		0.777 to 1.015		0.670 to 0.946		0.834 to 1.284	
P (log-rank test)	**.0419**		.0811		**.0093**		.75	
Progression-free survival*								
No. of events	322	298	281	263	189	146	92	117
Median, months	8.0	8.9	8.1	9.1	8.4	9.9	7.7	7.4
95% CI	7.6 to 9.0	8.0 to 9.5	7.5 to 9.0	8.0 to 9.6	7.4 to 9.2	9.0 to 11.3	7.3 to 9.2	6.1 to 8.0
Hazard ratio	0.851		0.855		0.696		1.171	
95% CI	0.726 to 0.998		0.721 to 1.013		0.558 to 0.867		0.887 to 1.544	
P (log-rank test)	**.0479**		.0709		**.0012**		.26	
Best overall response*								
Complete response	2	3	2	3	0	3	2	0
%	0.3	0.5	0.4	0.6		0.9	1.1	
Partial response	230	278	203	245	139	178	64	67
%	38.4	46.4	38.1	46.2	39.7	56.3	35.0	31.3
Stable disease	280	224	246	201	162	100	84	101
%	46.7	37.4	46.2	37.9	46.3	31.6	45.9	47.2
Progressive disease	54	53	51	43	31	19	20	24
%	9.0	8.8	9.6	8.1	8.9	6.0	10.9	11.2
Not evaluable	33	41	31	38	18	16	13	22
%	5.5	6.8	5.8	7.2	5.1	5.1	7.1	10.3
Best overall response rate†, %	38.7	46.9	38.5	46.8	39.7	57.3	36.1	31.3
95% CI	34.8 to 42.8	42.9 to 51.0	34.3 to 42.7	42.5 to 51.1	34.6 to 45.1	51.6 to 62.8	29.1 to 43.5	25.2 to 38.0
Odds ratio	1.403		1.414		2.069		0.822	
95% CI	1.115 to 1.766		1.108 to 1.804		1.515 to 2.826		0.544 to 1.242	
P (CMH test)	**.0038**		**.0052**		**< .001**		.35	

NOTE. $P < .05$ for bold values.
Abbreviations: ITT, intention-to-treat; CRYSTAL, Cetuximab Combined with Irinotecan in First-Line Therapy for Metastatic Colorectal Cancer; FOLFIRI, irinotecan, leucovorin and fluorouracil; CMH, Cochran-Mantel-Haenszel.
*As assessed by an independent review committee for the primary confirmatory analysis.[1]
†Best overall response rate = (complete response + partial response).

corresponding arms of the subpopulations defined according to *KRAS* and *BRAF* mutation status (Table 1; Appendix Table A1, online only). Exposure to irinotecan and fluorouracil was similar for patients in both treatment groups in the ITT[1] and *KRAS* populations and within each treatment group, for patients with *KRAS* wild-type and mutant tumors. In the ITT population, poststudy chemotherapy (or subsequent EGFR-targeted therapy with or without chemotherapy) was received by 65.9% of patients (11.0%) in the cetuximab plus FOLFIRI group and 70.1% (29.7%) of those receiving FOLFIRI alone.

Overall Survival in the ITT Population

As of May 31, 2009, there were 487 deaths (81% of patients) in the cetuximab plus FOLFIRI group and 502 (84% of patients) in the FOLFIRI alone group. The addition of cetuximab to FOLFIRI resulted in a significant improvement in overall survival time, with the stratified HR for death 0.878 (95% CI, 0.774 to 0.995; $P = .0419$), and median survival times of 19.9 months compared with 18.6 months for FOLFIRI alone (Table 2, Fig 2A).

Subgroup Analysis According to Tumor KRAS *Mutation Status*

Mutations in *KRAS* codon 12 or 13 were detected in the tumor tissue of 397 of 1,063 patients (37%). Mutations were found more frequently in the tumor tissue of patients receiving cetuximab plus FOLFIRI (40%) compared with those receiving FOLFIRI alone (34%). The HRs for PFS and overall survival in the ITT and *KRAS* populations were comparable (Table 2).

Patients whose tumors were wild-type for *KRAS* who received cetuximab plus FOLFIRI had a significantly reduced risk of disease progression (median PFS, 9.9 *v* 8.4 months; HR, 0.696; $P = .0012$) significantly improved overall survival (median survival, 23.5 *v* 20.0 months; HR, 0.796; $P = .0093$) and significantly increased odds of response (best overall response rate 57.3% *v* 39.7%; odds ratio, 2.069; $P < .001$) compared with those who received FOLFIRI alone (Table 2, Fig 2B; Appendix Fig A1, online only). Evaluable patients in the cetuximab plus FOLFIRI group with *KRAS* wild-type tumors who developed early acne-like rash (n = 207) had prolonged median survival times (26.4 *v* 19.1 months) compared with those not experiencing early acne-like rash (n = 101).

In patients whose tumors carried mutations in *KRAS*, there was no evidence of a benefit associated with the addition of cetuximab to FOLFIRI in relation to PFS, overall survival, or best overall response. Patients in both treatment groups whose tumors carried mutations in *KRAS* appeared to have worse overall survival than those whose tumors were wild-type (Table 2, Fig 2B). Cox (PFS and overall survival) and logistic regression (best overall response) models were used to explore the relationship in the expanded *KRAS* population between the magnitude of treatment effect and tumor *KRAS* mutation status. Significant interactions in the updated data set were noted for PFS ($P = .0028$), overall survival ($P = .0463$), and best overall response ($P = .0005$).

The rate of surgery for metastasis and the rate of R0 resection were both higher in patients with *KRAS* wild-type tumors who received cetuximab plus FOLFIRI compared with FOLFIRI alone (7.9% *v* 4.6%; odds ratio, 1.823; 95% CI, 0.957 to 3.472; $P = .0633$ and 5.1% *v* 2.0%; odds ratio, 2.650; 95% CI, 1.083 to 6.490; $P = .0265$, respectively).

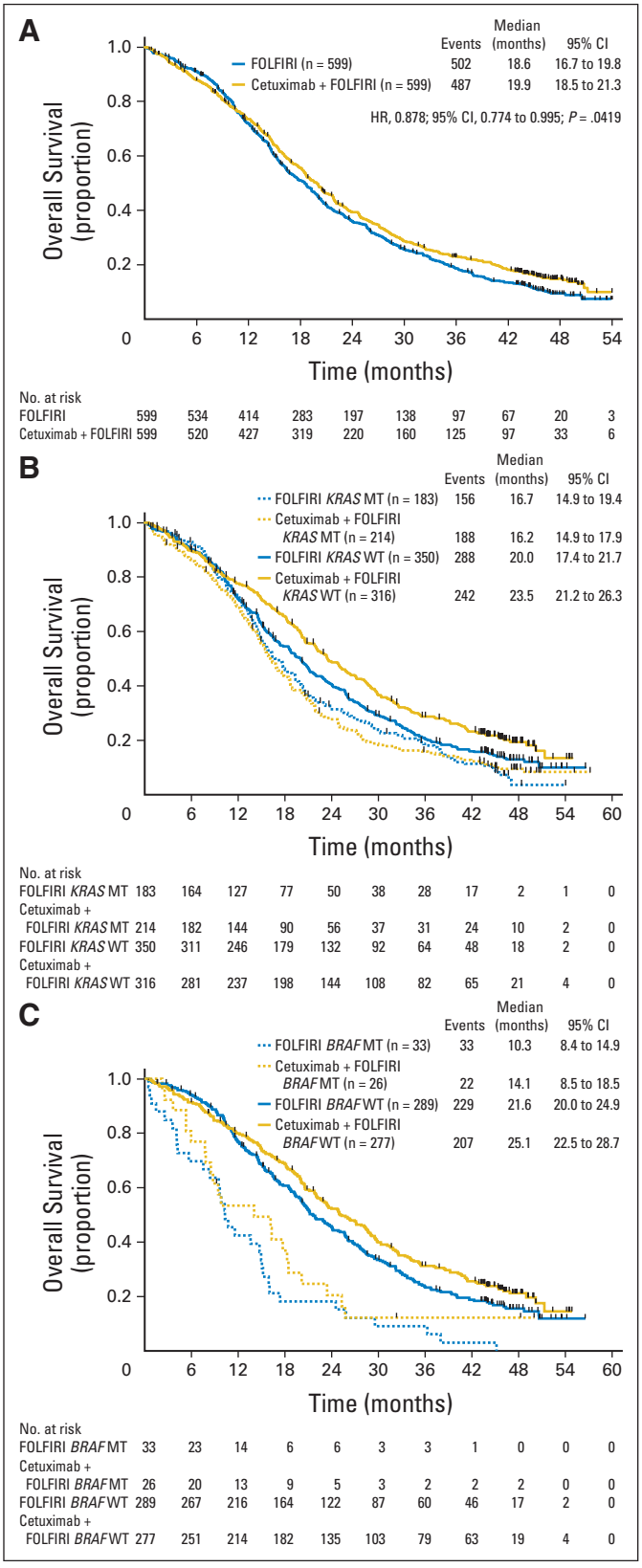

Fig 2. Kaplan-Meier plots for overall survival time according to treatment arm for (A) the intention-to-treat population; (B) patients in the *KRAS* population whose tumors were wild-type or mutant for *KRAS*; and (C) patients with *KRAS* wild-type disease according to tumor *BRAF* mutation status. FOLFIRI, irinotecan, leucovorin and fluorouracil; HR, hazard ratio; MT, mutant; WT, wild-type.

Poststudy chemotherapy was received by 66.1% versus 71.7% of patients with *KRAS* wild-type tumors in the cetuximab plus FOLFIRI compared with FOLFIRI alone group, respectively. Poststudy EGFR-targeted therapy (with or without chemotherapy) was administered to 10.8% versus 30.9% of patients in these groups, respectively.

Clinical Impact of Tumor BRAF *Mutation in Patients With* KRAS *Wild-Type Tumors*

BRAF V600E mutations were detected in 60 (6%) of 999 tumor samples evaluable for both *BRAF* and *KRAS*. In all but one case, these mutations were identified in tumors which were wild-type for *KRAS*. The impact of *BRAF* tumor mutation status in relation to the efficacy of cetuximab plus FOLFIRI was examined in the population of patients with *KRAS* wild-type disease (n = 625). Patients whose tumors were wild-type for both genes who received cetuximab plus FOLFIRI had a significantly reduced risk of disease progression (HR, 0.637; $P = .0013$) and significantly increased odds of response (odds ratio, 2.175; $P < .001$) compared with those who received FOLFIRI alone (Table 3). The overall survival benefit in this group was no longer significant (HR, 0.830; $P = .0547$; Appendix Fig A1, Table A1). In patients whose tumors were *KRAS* wild-type/*BRAF* mutant, improvements in PFS (median 8.0 *v* 5.6 months; HR, 0.934; $P = .87$) and overall survival (median, 14.1 *v* 10.3 months; HR, 0.908; $P = .74$) associated with the addition of cetuximab to FOLFIRI did not reach statistical significance (Table 3; Appendix Fig A1). There was no evidence of an independent treatment by tumor *BRAF* mutation status interaction. Thus, with the current data set, *BRAF* mutation status cannot be shown to be predictive of treatment effects of cetuximab plus FOLFIRI. *BRAF* V600E mutation indicated poor prognosis in patients with *KRAS* wild-type disease in both treatment groups, with those whose tumors carried *BRAF* mutations having a worse outcome for all efficacy end points compared with those whose tumors were wild-type (Table 3; Fig 2C).

Safety

The most common grade 3/4 AE in the safety population was neutropenia, which occurred in 28.2% of patients receiving cetuximab plus FOLFIRI and 24.9% of those receiving FOLFIRI alone. As expected, the incidences of skin reactions, diarrhea, and infusion-related reactions were somewhat higher in patients in the cetuximab plus FOLFIRI compared with the FOLFIRI alone group. Toxicity profiles according to treatment arm were comparable for the safety and *KRAS*

Table 3. Efficacy Data for Patients With *KRAS* Wild-Type Tumors According to Tumor *BRAF* Mutation Status

	KRAS Wild-Type/*BRAF* Wild-Type (n = 566)		*KRAS* Wild-Type/*BRAF* Mutant (n = 59)	
Parameter	FOLFIRI (n = 289)	Cetuximab + FOLFIRI (n = 277)	FOLFIRI (n = 33)	Cetuximab + FOLFIRI (n = 26)
Overall survival				
No. of events	229	207	33	22
Median, months	21.6	25.1	10.3	14.1
95% CI	20.0 to 24.9	22.5 to 28.7	8.4 to 14.9	8.5 to 18.5
Hazard ratio	0.830		0.908	
95% CI	0.687 to 1.004		0.507 to 1.624	
P (log-rank test)	.0547		.74	
Progression-free survival*				
No. of events	153	123	20	14
Median, months	8.8	10.9	5.6	8.0
95% CI	7.6 to 9.4	9.4 to 11.8	3.5 to 8.1	3.6 to 9.1
Hazard ratio	0.673		0.934	
95% CI	0.528 to 0.858		0.425 to 2.056	
P (log-rank test)	**.0013**		.87	
Best overall response*				
Complete response	0	3	0	0
%		1.1		
Partial response	123	166	5	5
%	42.6	59.9	15.2	19.2
Stable disease	135	80	16	17
%	46.7	28.9	48.5	65.4
Progressive disease	18	14	8	2
%	6.2	5.1	24.2	7.7
Not evaluable	13	14	4	2
%	4.5	5.1	12.1	7.7
Best overall response rate†, %	42.6	61.0	15.2	19.2
95% CI	36.8 to 48.5	55.0 to 66.8	5.1 to 31.9	6.6 to 39.4
Odds ratio	2.175		1.084	
95% CI	1.551 to 3.051		0.264 to 4.446	
P (CMH test)	**< .001**		.91	

NOTE. $P < .05$ for bold values.
Abbreviations: FOLFIRI, irinotecan, leucovorin and fluorouracil; CMH, Cochran-Mantel-Haenszel.
*As assessed by an independent review committee for the primary confirmatory analysis.[1]
†Best overall response rate = (complete response + partial response).

Table 4. Most Common Grade 3/4 Adverse Events in the CRYSTAL Safety Population and Frequencies in *KRAS* Subpopulations

	CRYSTAL Safety Population (n = 1,202)*				*KRAS* Population (n = 1,064)				*KRAS* Population							
									KRAS Wild-Type (n = 667)				*KRAS* Mutant (n = 397)			
	FOLFIRI (n = 602)		Cetuximab + FOLFIRI (n = 600)		FOLFIRI (n = 533)		Cetuximab + FOLFIRI (n = 531)		FOLFIRI (n = 350)		Cetuximab + FOLFIRI (n = 317)		FOLFIRI (n = 183)		Cetuximab + FOLFIRI (n = 214)	
Adverse Event	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Any	367	61.0	476	79.3	323	60.6	421	79.3	211	60.3	257	81.1	112	61.2	164	76.6
MedDRA preferred term																
Neutropenia	150	24.9	169	28.2	133	25.0	150	28.2	83	23.7	97	30.6	50	27.3	53	24.8
Leukopenia	32	5.3	43	7.2	27	5.1	37	7.0	17	4.9	25	7.9	10	5.5	12	5.6
Diarrhea	63	10.5	94	15.7	55	10.3	79	14.9	35	10.0	52	16.4	20	10.9	27	12.6
Vomiting	30	5.0	28	4.7	25	4.7	24	4.5	16	4.6	13	4.1	9	4.9	11	5.1
Fatigue	28	4.7	32	5.3	23	4.3	30	5.6	20	5.7	14	4.4	3	1.6	16	7.5
Rash	0		49	8.2	0		44	8.3	0		28	8.8	0		16	7.5
Dermatitis acneiform	0		32	5.3	0		28	5.3	0		16	5.0	0		12	5.6
Special adverse events																
Skin reactions†																
All	1	0.2	117	19.5	1	0.2	107	20.2	1	0.3	67	21.1	0		40	18.7
Acne-like rash	0		97	16.2	0		87	16.4	0		52	16.4	0		35	16.4
Infusion-related reactions	0		14	2.3	0		12	2.3	0		5	1.6	0		7	3.3

Abbreviations: CRYSTAL, Cetuximab Combined with Irinotecan in First-Line Therapy for Metastatic Colorectal Cancer; FOLFIRI, irinotecan, leucovorin, and fluorouracil; MedDRA, Medical Dictionary for Regulatory Activities (version 10.0).

*In addition to the 1,198 patients of the intention-to-treat population, the CRYSTAL study safety population included four other patients (one included in the *KRAS* wild-type group) who were treated but did not undergo random assignment.

†No grade 4 reactions in relation to any MedDRA preferred term included in skin reactions composite categories.

populations and for the subpopulations with *KRAS* wild-type and mutant tumors (Table 4).

DISCUSSION

With knowledge of the predictive value of tumor *KRAS* mutation status in relation to the efficacy of cetuximab[4-7] and the EGFR immunoglobulin G2 monoclonal antibody panitumumab[17] in pretreated patients, the CRYSTAL study data were reanalyzed according to tumor *KRAS* status. Clinical material from which tumor DNA could be successfully analyzed was initially available for a subset of 45% of patients. Subsequently, by using improved ascertainment approaches, the number of patients for whom tumor *KRAS* mutation status could be determined was approximately doubled to 89%. It was anticipated that the increase in the number of patients evaluable for the status of this biomarker and the increased follow-up time for survival would allow more accurate assessment of the impact of tumor *KRAS* mutation status. We report an updated analysis in the larger cohort, as well as novel data on the impact of tumor *BRAF* mutations on clinical outcome.

The updated analysis indicated that the addition of cetuximab to FOLFIRI significantly improved overall survival in the first-line treatment of patients with mCRC compared with FOLFIRI alone. A significant interaction between tumor *KRAS* mutation status and treatment effect was demonstrated for all key efficacy end points, and analyses within the individual treatment arms indicated that the clinical benefit conferred by cetuximab was limited to patients with *KRAS* wild-type disease. The median survival time of 23.5 months for patients with *KRAS* wild-type mCRC who received cetuximab plus FOLFIRI is among the longest reported for a randomized phase III study in this setting. It should also be noted that the HRs for overall survival favoring the cetuximab plus FOLFIRI group were similar in both the initial and updated data sets for patients with *KRAS* wild-type tumors (0.84 and 0.796, respectively). A further factor that should be considered is that although the overall survival results were most likely impacted by an unbalanced administration of EGFR-targeting agents as poststudy therapy, a clinically relevant survival benefit was nevertheless demonstrated. As to which of the currently approved targeted agents is the most effective when used in combination with FOLFIRI as first-line treatment for patients with mCRC, or particular subgroups of such patients, the difficulties inherent in cross-study comparisons preclude the formulation of a definitive answer.

The CRYSTAL study results are consistent with the updated data from the randomized phase II Oxaliplatin and Cetuximab in First-Line Treatment of Metastatic Colorectal Cancer (OPUS) study, which confirmed *KRAS* tumor mutation status as an effective biomarker for the efficacy of cetuximab plus oxaliplatin, leucovorin, and infusional fluorouracil in the first-line treatment of patients with *KRAS* wild-type mCRC.[18] These data are therefore entirely consistent with the revised guidance from regulatory and advisory authorities concerning the administration of cetuximab only to patients with *KRAS* wild-type mCRC.[19-22] They confirm *KRAS* tumor mutation status as a powerful predictive biomarker in this setting in relation to the clinical efficacy of cetuximab combined with standard first-line chemotherapy. It is anticipated that further investigations will yield additional clinical and molecular markers enabling the accurate prediction of which patients with *KRAS* wild-type mCRC are most likely to derive a clinical benefit from cetuximab treatment. It was in this context that tumor *BRAF* mutation status was examined in this study.

Patients in both arms with *KRAS* wild-type disease whose tumors carried *BRAF* V600E mutations were found to have worse outcomes for all efficacy end points compared with those with tumors wild-type at this codon, thereby confirming this mutation as a strong indicator of poor prognosis in patients receiving chemotherapy alone. These

data are consistent with the biomarker analysis of patient tissues from the randomized Fluorouracil, Oxaliplatin, and Irinotecan: Use and Sequencing (FOCUS) and CAIRO2 studies, which demonstrated that tumor *BRAF* mutation was a negative prognostic marker for overall survival in patients with mCRC.[12,23] Indeed, this strong prognostic effect may explain at least in part why previous single-arm retrospective mCRC analyses have been interpreted as perhaps indicating that EGFR-targeted therapy is ineffective in patients with *BRAF* mutant tumors.[11,24,25] In this study, although there was a marginal trend toward improved PFS and overall survival in patients with *KRAS* wild-type/*BRAF* mutant tumors receiving cetuximab plus FOLFIRI compared with FOLFIRI alone, whether this biomarker is a negative predictor in relation to cetuximab benefit could not be definitively addressed given the relatively small number of patients with *BRAF* mutations. Other candidate biomarkers which may have predictive utility in this setting include high level expression in tumors of the EGFR ligands amphiregulin and epiregulin,[26-28] and tumor mutation status of the *PIK3CA*[13,29,30] gene.

In summary, this study has demonstrated unequivocally that the addition of cetuximab to FOLFIRI as first-line therapy for mCRC improves overall survival compared with FOLFIRI alone in patients whose tumors are wild-type for *KRAS*. *BRAF* tumor mutation status is a strong indicator of poor prognosis and should be considered as a stratification factor for future mCRC studies.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Although all authors completed the disclosure declaration, the following author(s) indicated a financial or other interest that is relevant to the subject matter under consideration in this article. Certain relationships marked with a "U" are those for which no compensation was received; those relationships marked with a "C" were compensated. For a detailed description of the disclosure categories, or for more information about ASCO's conflict of interest policy, please refer to the Author Disclosure Declaration and the Disclosures of Potential Conflicts of Interest section in Information for Contributors.

Employment or Leadership Position: Michael Schlichting, Merck KGaA (C); Angela Zubel, Merck KGaA (C); Ilhan Celik, Merck KGaA (C) **Consultant or Advisory Role:** Eric Van Cutsem, Merck KGaA (C), Roche (C); Gunnar Folprecht, Merck KGaA (C), Genentech (C); Stefano Cascinu, Merck KGaA (C); David Cunningham, Merck KGaA (C); Philippe Rougier, Merck Serono (C), Pfizer (C) **Stock Ownership:** None **Honoraria:** Claus-Henning Köhne, Merck KGaA, Pfizer, Roche; Gunnar Folprecht, Merck KGaA, Amgen, Roche; David Cunningham, Merck KGaA; Sabine Tejpar, Merck Serono; Fortunato Ciardiello, Merck KGaA, Roche **Research Funding:** Eric Van Cutsem, Merck KGaA, Pfizer, Amgen, sanofi-aventis, Roche; Claus-Henning Köhne, Merck KGaA, Pfizer, Roche; Gunnar Folprecht, Merck KGaA; David Cunningham, Merck KGaA, Pfizer; Sabine Tejpar, Merck Serono; Fortunato Ciardiello, Merck KGaA, Roche, AstraZeneca **Expert Testimony:** None **Other Remuneration:** None

AUTHOR CONTRIBUTIONS

Conception and design: Eric Van Cutsem, Claus-Henning Köhne, Sabine Tejpar, Michael Schlichting, Ilhan Celik, Philippe Rougier
Provision of study materials or patients: Eric Van Cutsem, Claus-Henning Köhne, Gunnar Folprecht, Philippe Rougier, Fortunato Ciardiello
Collection and assembly of data: Eric Van Cutsem, István Láng, Gunnar Folprecht, Marek P. Nowacki, Stefano Cascinu, David Cunningham, Michael Schlichting, Ilhan Celik, Philippe Rougier, Fortunato Ciardiello
Data analysis and interpretation: Eric Van Cutsem, Claus-Henning Köhne, Gunnar Folprecht, Igor Shchepotin, Joan Maurel, Michael Schlichting, Angela Zubel, Ilhan Celik, Philippe Rougier, Fortunato Ciardiello
Manuscript writing: All authors
Final approval of manuscript: All authors

REFERENCES

1. Van Cutsem E, Köhne CH, Hitre E, et al: Cetuximab and chemotherapy as initial treatment for metastatic colorectal cancer. N Engl J Med 360:1408-1417, 2009

2. Vermorken JB, Mesia R, Rivera F, et al: Platinum-based chemotherapy plus cetuximab in head and neck cancer. N Engl J Med 359:1116-1127, 2008

3. Pirker R, Pereira JR, Szczesna A, et al: Cetuximab plus chemotherapy in patients with advanced non-small-cell lung cancer (FLEX): An open-label randomised phase III trial. Lancet 373:1525-1531, 2009

4. De Roock W, Piessevaux H, De Schutter J, et al: KRAS wild-type state predicts survival and is associated to early radiological response in metastatic colorectal cancer treated with cetuximab. Ann Oncol 19:508-515, 2008

5. Di Fiore F, Blanchard F, Charbonnier F, et al: Clinical relevance of KRAS mutation detection in metastatic colorectal cancer treated by cetuximab plus chemotherapy. Br J Cancer 96:1166-1169, 2007

6. Lièvre A, Bachet JB, Boige V, et al: KRAS mutations as an independent prognostic factor in patients with advanced colorectal cancer treated with cetuximab. J Clin Oncol 26:374-379, 2008

7. Lièvre A, Bachet JB, Le Corre D, et al: KRAS mutation status is predictive of response to cetuximab therapy in colorectal cancer. Cancer Res 66:3992-3995, 2006

8. Bokemeyer C, Bondarenko I, Makhson A, et al: Fluorouracil, leucovorin, and oxaliplatin with and without cetuximab in the first-line treatment of metastatic colorectal cancer. J Clin Oncol 27:663-671, 2009

9. Karapetis CS, Khambata-Ford S, Jonker DJ, et al: K-ras mutations and benefit from cetuximab in advanced colorectal cancer. N Engl J Med 359:1757-1765, 2008

10. Ji H, Wang Z, Perera SA, et al: Mutations in BRAF and KRAS converge on activation of the mitogen-activated protein kinase pathway in lung cancer mouse models. Cancer Res 67:4933-4939, 2007

11. Loupakis F, Ruzzo A, Cremolini C, et al: KRAS codon 61, 146 and BRAF mutations predict resistance to cetuximab plus irinotecan in KRAS codon 12 and 13 wild-type metastatic colorectal cancer. Br J Cancer 101:715-721, 2009

12. Richman SD, Seymour MT, Chambers P, et al: KRAS and BRAF mutations in advanced colorectal cancer are associated with poor prognosis but do not preclude benefit from oxaliplatin or irinotecan: Results from the MRC FOCUS trial. J Clin Oncol 27:5931-5937, 2009

13. Souglakos J, Philips J, Wang R, et al: Prognostic and predictive value of common mutations for treatment response and survival in patients with metastatic colorectal cancer. Br J Cancer 101:465-472, 2009

14. Baldus SE, Schaefer KL, Engers R, et al: Prevalence and heterogeneity of KRAS, BRAF, and PIK3CA mutations in primary colorectal adenocarcinomas and their corresponding metastases Clin Cancer Res 16:790-799, 2010

15. Kaplan EL, Meier P: Nonparametric estimation from incomplete observations. J Am Stat Assoc 53:457-481, 1958

16. Gatzemeier U, von Pawel J, Vynnychenko I, et al: First-cycle rash and survival in patients with advanced non-small-cell lung cancer receiving cetuximab in combination with first-line chemotherapy: A subgroup analysis of data from the FLEX phase 3 study. Lancet Oncol 12:30-37, 2011

17. Amado RG, Wolf M, Peeters M, et al: Wild-type KRAS is required for panitumumab efficacy in patients with metastatic colorectal cancer. J Clin Oncol 26:1626-1634, 2008

18. Bokemeyer C, Bondarenko I, Hartmann JT, et al: Efficacy according to biomarker status of cetuximab plus FOLFOX-4 as first-line treatment for metastatic colorectal cancer. The OPUS study. Ann Oncol [epub ahead of print on January 12, 2011]

19. European Medicines Agency: Erbitux European Public Assessment Report (Updated 14 July 2009). http://www.emea.europa.eu/humandocs/Humans/EPAR/erbitux/erbitux.htm

20. US Food and Drug Administration: Full Prescribing Information - Erbitux: Http://www.accessdata.fda.gov/drugsatfda_docs/label/2009/125084s168lbl.pdf

21. Allegra CJ, Jessup JM, Somerfield MR, et al: American Society of Clinical Oncology provisional clinical opinion: Testing for KRAS gene mutations in

patients with metastatic colorectal carcinoma to predict response to anti-epidermal growth factor receptor monoclonal antibody therapy. J Clin Oncol 27:2091-2096, 2009

22. Van Cutsem E, Nordlinger B, Cervantes A: Advanced colorectal cancer: ESMO Clinical Practice Guidelines for treatment. Ann Oncol 21:v93-v97, 2010 (suppl 5)

23. Tol J, Dijkstra JR, Klomp M, et al: Markers for EGFR pathway activation as predictor of outcome in metastatic colorectal cancer patients treated with or without cetuximab. Eur J Cancer 46:1997-2009, 2010

24. Di Nicolantonio F, Martini M, Molinari F, et al: Wild-type BRAF is required for response to panitumumab or cetuximab in metastatic colorectal cancer. J Clin Oncol 26:5705-5712, 2008

25. Bardelli A, Siena S: Molecular mechanisms of resistance to cetuximab and panitumumab in colorectal cancer. J Clin Oncol 28:1254-1261, 2010

26. Khambata-Ford S, Garrett CR, Meropol NJ, et al: Expression of epiregulin and amphiregulin and K-ras mutation status predict disease control in metastatic colorectal cancer patients treated with cetuximab. J Clin Oncol 25:3230-3237, 2007

27. Jacobs B, De Roock W, Piessevaux H, et al: Amphiregulin and epiregulin mRNA expression in primary tumors predicts outcome in metastatic colorectal cancer treated with cetuximab. J Clin Oncol 27:5068-5074, 2009

28. Tabernero J, Cervantes A, Rivera F, et al: Pharmacogenomic and pharmacoproteomic studies of cetuximab in metastatic colorectal cancer: Biomarker analysis of a phase I dose-escalation study. J Clin Oncol 28:1181-1189, 2010

29. Sartore-Bianchi A, Martini M, Molinari F, et al: PIK3CA mutations in colorectal cancer are associated with clinical resistance to EGFR-targeted monoclonal antibodies. Cancer Res 69: 1851-1857, 2009

30. De Roock W, Claes B, Bernasconi D, et al: Effects of KRAS, BRAF, NRAS, and PIK3CA mutations on the efficacy of cetuximab plus chemotherapy in chemotherapy-refractory metastatic colorectal cancer: A retrospective consortium analysis Lancet Oncol 11: 753-762, 2010

Acknowledgment

We thank Patrick Lebrun for his contribution to this study; Christopher Stroh, Merck KGaA, for his contribution in relation to the biomarker analyses; and Tobias Haas, Merck KGaA, for carrying out the *KRAS* and *BRAF* mutation detection assays. Editorial assistance in the preparation of this article was provided by Jim Heighway, PhD, Cancer Communications & Consultancy Ltd, funded by Merck KGaA, Darmstadt, Germany.

Appendix

Table A1. Patient and Disease Characteristics at Baseline for *KRAS* Wild-Type Patients According to *BRAF* Mutation Status

Characteristic	*KRAS* Wild-Type/*BRAF* Wild-Type (n = 566)		*KRAS* Wild-Type/*BRAF* Mutant (n = 59)	
	FOLFIRI (n = 289)	Cetuximab + FOLFIRI (n = 277)	FOLFIRI (n = 33)	Cetuximab + FOLFIRI (n = 26)
Sex, %				
Male	61.2	63.5	54.5	57.7
Female	38.8	36.5	45.5	42.3
Age, years				
Median	59	60	58	64.5
Range	19-84	24-79	25-75	34-79
Region, %				
Western Europe	44.3	43.7	57.6	73.1
Eastern Europe	38.4	40.1	27.3	11.5
Outside Europe	17.3	16.2	15.2	15.4
ECOG PS, %				
0	60.2	60.3	54.5	38.5
1	36.7	35.7	36.4	61.5
2	3.1	4.0	9.1	-
Laboratory values, %				
Lactate dehydrogenase > ULN	43.3	44.4	30.3	30.8
Alkaline phosphatase ≥ 300 U/L	11.1	9.4	12.1	11.5
Leukocyte count > 10,000/μL	15.9	15.2	15.2	15.4
Prior adjuvant chemotherapy, %	21.5	27.1	18.2	15.4
Metastases, %				
At one or two sites	85.5	88.4	72.7	80.8
Confined to liver	21.5	20.6	12.1	34.6

Abbreviations: FOLFIRI, irinotecan, leucovorin, and fluorouracil; ECOG PS, Eastern Cooperative Oncology Group performance status; ULN, upper limit of normal.

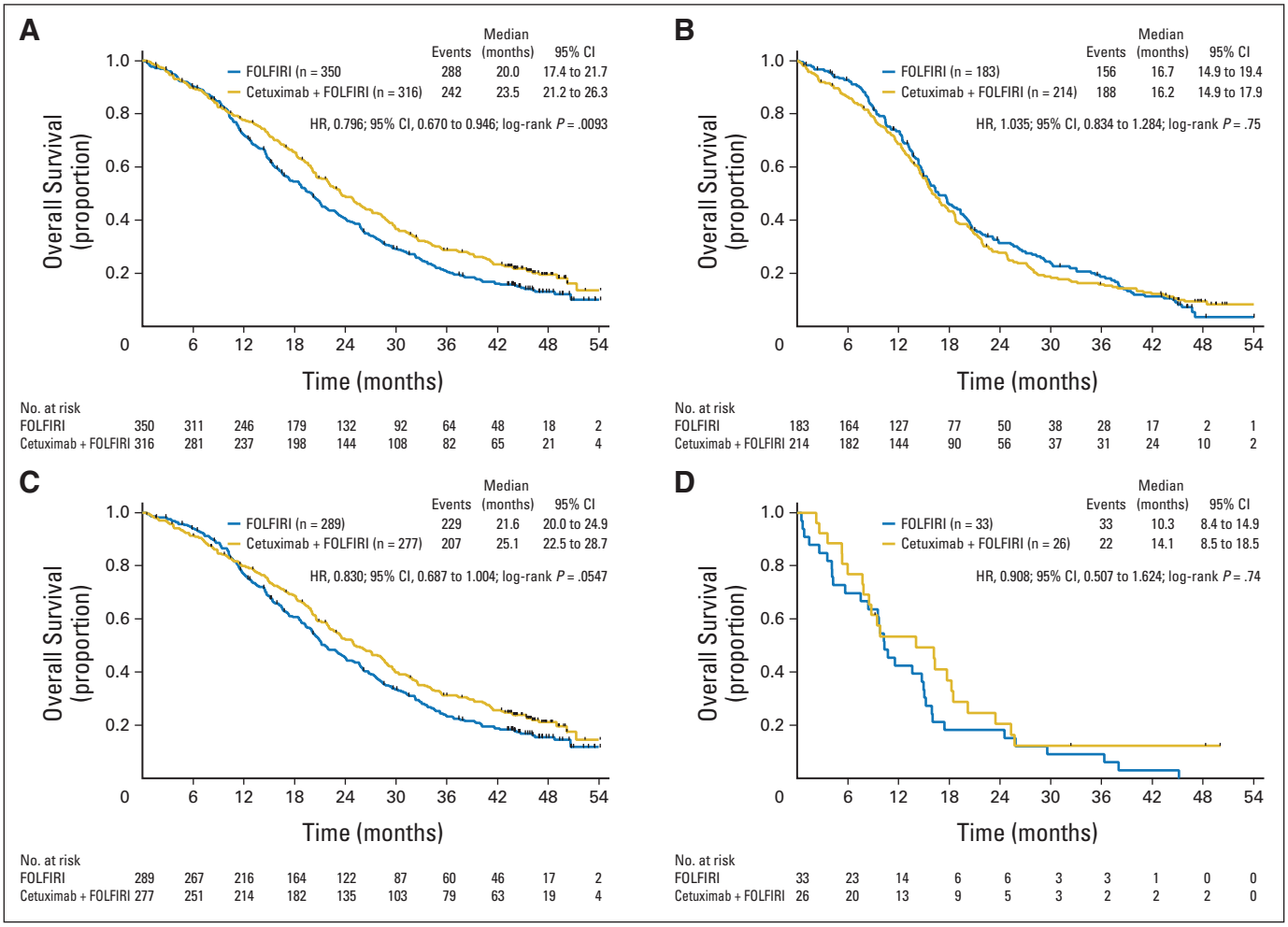

Fig A1. Overall survival according to treatment arm and tumor mutation status. (A) Patients with *KRAS* wild-type tumors; (B) patients with *KRAS* mutant tumors; (C) patients with *KRAS* wild-type/*BRAF* wild-type tumors; (D) patients with *KRAS* wild-type/*BRAF* mutant tumors. FOLFIRI, irinotecan, leucovorin and fluorouracil; HR, hazard ratio.

