

Fluorouracil, Leucovorin, and Oxaliplatin With and Without Cetuximab in the First-Line Treatment of Metastatic Colorectal Cancer

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ABSTRACT

Purpose

This randomized study assessed whether the best overall response rate (ORR) of cetuximab combined with oxaliplatin, leucovorin, and fluorouracil (FOLFOX-4) was superior to that of FOLFOX-4 alone as first-line treatment for metastatic colorectal cancer. The influence of *KRAS* mutation status was investigated.

Patients and Methods

Patients received cetuximab (400 mg/m² initial dose followed by 250 mg/m²/wk thereafter) plus FOLFOX-4 (oxaliplatin 85 mg/m² on day 1, plus leucovorin 200 mg/m² and fluorouracil as a 400 mg/m² bolus followed by a 600 mg/m² infusion during 22 hours on days 1 and 2; n = 169) or FOLFOX-4 alone (n = 168). Treatment was continued until disease progression or unacceptable toxicity. *KRAS* mutation status was assessed in the subset of patients with assessable tumor samples (n = 233).

Results

The confirmed ORR for cetuximab plus FOLFOX-4 was higher than with FOLFOX-4 alone (46% v 36%). A statistically significant increase in the odds for a response with the addition of cetuximab to FOLFOX-4 could not be established (odds ratio = 1.52; *P* = .064). In patients with *KRAS* wild-type tumors, the addition of cetuximab to FOLFOX-4 was associated with a clinically significant increased chance of response (ORR = 61% v 37%; odds ratio = 2.54; *P* = .011) and a lower risk of disease progression (hazard ratio = 0.57; *P* = .0163) compared with FOLFOX-4 alone. Cetuximab plus FOLFOX-4 was generally well tolerated.

Conclusion

KRAS mutational status was shown to be a highly predictive selection criterion in relation to the treatment decision regarding the addition of cetuximab to FOLFOX-4 for previously untreated patients with metastatic colorectal cancer.

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INTRODUCTION

The epidermal growth factor receptor (EGFR) is a clinically validated anticancer molecular target that is expressed in the majority of colorectal tumors.¹⁻⁵ Cetuximab (Erbix, developed by Merck KGaA [Darmstadt, Germany], under license from Imclone New York, NY) is an immunoglobulin G1 monoclonal antibody that specifically targets EGFR with high affinity, competitively inhibiting endogenous ligand binding and ligand-dependent downstream signaling.^{6,7} Binding to the tumor cell also initiates antibody-dependent cell-mediated cytotoxicity.⁸⁻¹⁰ Randomized phase II and III metastatic colorectal cancer (mCRC)

studies have demonstrated the efficacy and tolerability of cetuximab, as monotherapy^{2,11} or in combination with irinotecan,^{2,12} after the failure of previous chemotherapy regimens including irinotecan and/or oxaliplatin. Furthermore, in the first-line setting, building on promising phase II data,¹³ the phase III Cetuximab Combined With Irinotecan in First-Line Therapy for Metastatic Colorectal Cancer (CRYSTAL) study has shown that the addition of cetuximab to infusional fluorouracil (FU)/leucovorin (LV)/irinotecan (FOLFIRI) significantly improves the progression-free survival (PFS) time, response rate, and R0 resection rate in mCRC patients, compared with FOLFIRI alone.¹⁴

Phase II studies examining the activity and tolerability of cetuximab combined with oxaliplatin and FU/LV (FOLFOX-4) have provided encouraging results.^{15,16} To further evaluate this combination, the Oxaliplatin and Cetuximab in First-Line Treatment of mCRC (OPUS) study compared the efficacy and safety of cetuximab plus FOLFOX-4 with FOLFOX-4 alone as first-line therapy for mCRC.

The definition of relevant molecular characteristics of an individual tumor (biomarker evaluation) will increasingly enable the selection of patients most likely to benefit from particular treatments.¹⁷ A recent subgroup analysis of a randomized phase III study of the immunoglobulin G2 EGFR-targeting monoclonal antibody panitumumab in patients with chemotherapy-refractory mCRC suggested a lack of clinical activity in patients whose tumors had activating mutations at codon 12 or 13 of the *KRAS* gene.¹⁸ Small retrospective studies in chemotherapy-refractory patients have also suggested that clinical responses to cetuximab are confined to those mCRC patients (approximately 55% to 70%) whose tumors are wild type for *KRAS*.¹⁹⁻²² This assertion has recently been confirmed in subgroup analyses of the randomized phase III CRYSTAL and CO.17 studies.^{23,24} A subgroup analysis of the OPUS study therefore investigated whether tumor *KRAS* mutation status was predictive of a clinical response to cetuximab plus FOLFOX-4.

PATIENTS AND METHODS

Patient Eligibility

Patients were eligible for inclusion if they were ≥ 18 years old; had a histologically confirmed, first-occurrence of a nonresectable, EGFR-expressing mCRC with at least one radiologically measurable lesion; a life expectancy of ≥ 12 weeks; an Eastern Cooperative Oncology Group Performance Status (ECOG PS) ≤ 2 ; and adequate hepatic, renal, and bone marrow function. Patients were ineligible if they were pregnant, had a history of previous exposure to EGFR-targeted therapy or previous chemotherapy (except adjuvant treatment) for mCRC, or had uncontrolled severe organ or metabolic dysfunction. The study was approved by relevant ethics committees and was conducted in accordance with the Declaration of Helsinki. All patients provided written, informed consent.

Study Design

This was an open-label, randomized, multicenter phase II study comparing the efficacy and safety of cetuximab combined with FOLFOX-4 versus FOLFOX-4 alone in the first-line treatment of EGFR-expressing mCRC. EGFR expression was determined using a DakoCytomation pharmDx immunohistochemistry kit (Dako, Glostrup, Denmark), with one tumor cell staining to any intensity being the minimum criterion required to confirm expression. Randomization (1:1) was carried out using a stratified permuted-block procedure, with ECOG PS (0 and 1 v 2) as a stratification factor.

On day 1 of a 14-day treatment cycle, patients received cetuximab (initial dose 400 mg/m² infused during 2 hours, and 250 mg/m² weekly during 1 hour thereafter) followed after 1 hour by FOLFOX-4 (oxaliplatin 85 mg/m² on day 1, infused during 2 hours; LV 200 mg/m², infused during 2 hours, followed by FU as a 400 mg/m² intravenous bolus then a 600 mg/m² infusion during 22 hours on days 1 and 2) or FOLFOX-4 alone, until the occurrence of progressive disease (PD) or unacceptable toxicity. In the case of unacceptable toxicity or intolerance to FU/LV, oxaliplatin, or cetuximab, the agent responsible could be stopped and the patient could continue with the other study medication(s). Radiologic assessment of response was carried out every 8 weeks until PD or withdrawal for any reason.

The primary objective was to assess whether the best confirmed overall response rate of cetuximab plus FOLFOX-4 was superior to that of FOLFOX-4 alone. Tumor response including disease progression was assessed by an independent review committee according to modified WHO

criteria. The independent review committee conducted a blinded review of images and clinical data using a common set of prespecified criteria. The data cutoff for the primary analysis of response was chosen to be 20 weeks after the last randomly assigned patient started study treatment (August 4, 2006) so that all patients could have at least two 8-week evaluations. Secondary objectives included treatment comparisons of the rate of curative metastatic surgery, duration of response, disease control rate, PFS time, OS time, and safety, all of which were based on data from a longer follow-up time (March 1, 2007). A retrospective subgroup analysis investigated associations between PFS and response rate and the *KRAS* mutation status of tumors.

Statistical Methods and Considerations

The primary efficacy analysis was performed on the intention-to-treat (ITT) population, defined as all randomly assigned patients who received at least one dose of study treatment. Assuming a difference in the rate of best confirmed response of at least 20% between the two treatments (ie, approximately 70% with cetuximab plus FOLFOX-4 and 50% with FOLFOX-4 alone for the stratum with ECOG PS 0 to 1, and 66% and 45%, respectively, for the ECOG PS 2 stratum), the Cochran-Mantel-Haenszel (CMH)^{25,26} odds ratio (OR) over the strata was expected to be 2.33. A sample size of approximately 146 patients per group was therefore calculated as necessary to detect an odds ratio of 2.33 at level $\alpha = .05$, with a

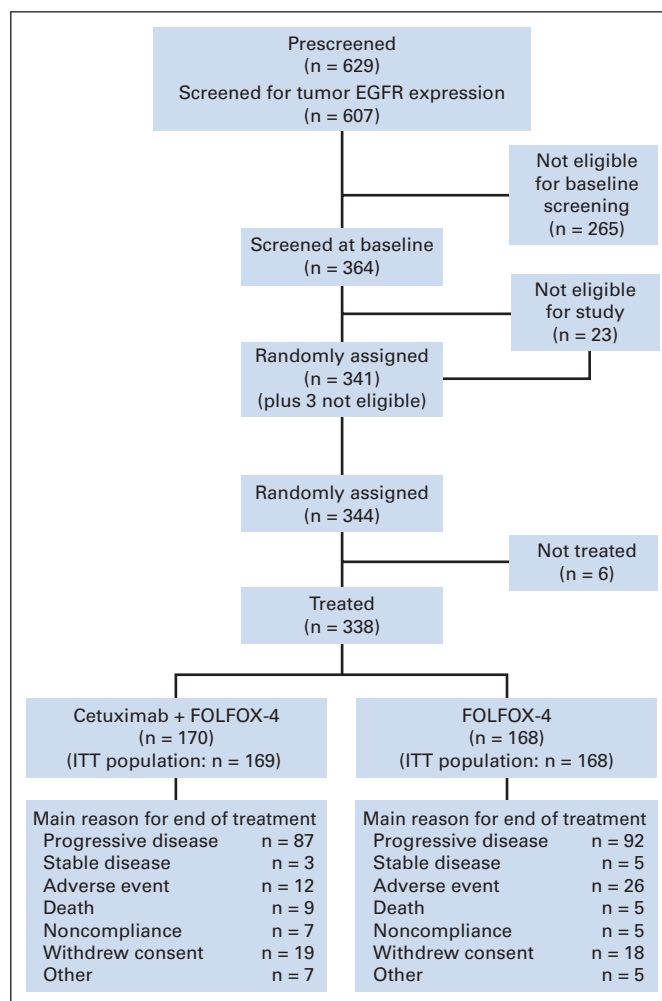


Fig 1. Disposition of subjects at the time of the data cutoff March 1, 2007. EGFR, epidermal growth factor receptor; FOLFOX-4, oxaliplatin, leucovorin, and fluorouracil; ITT, intention to treat.

power of 90%. With the exception of the test of the null hypothesis of $OR = 1$ over the entire sample population, all further efficacy analyses were exploratory and P values were not adjusted for the multiplicity of statistical tests. Time-to-event data were analyzed using the Kaplan-Meier method.²⁷ All analyses were repeated by *KRAS* mutation status in those ITT patients whose tumors were available and provided an assessable DNA sample (*KRAS* population).

KRAS Mutation Detection

Tumor DNA was extracted from formaldehyde-fixed paraffin-embedded tissues, and screened for the presence of *KRAS* codon 12 and 13 mutations using a polymerase chain reaction (PCR) clamping and melting curve technique adapted from Chen et al²⁸ (full methods information is listed in the Appendix, online only). Briefly, a one-step Lightcycler PCR reaction (Light-Mix, k-ras Gly12; TIB MOLBIOL, Berlin, Germany) incorporated a codon 12

Table 1. Patient and Disease Characteristics at Baseline

Characteristic	ITT Population (n = 337)				<i>KRAS</i> Population (n = 233)				<i>KRAS</i> Wild Type (n = 134)				<i>KRAS</i> Mutant (n = 99)			
	FOLFOX-4 (n = 168)		Cetuximab + FOLFOX-4 (n = 169)		FOLFOX-4 (n = 120)		Cetuximab + FOLFOX-4 (n = 113)		FOLFOX-4 (n = 73)		Cetuximab + FOLFOX-4 (n = 61)		FOLFOX-4 (n = 47)		Cetuximab + FOLFOX-4 (n = 52)	
	No. of Patients	%	No. of Patients	%	No. of Patients	%	No. of Patients	%	No. of Patients	%	No. of Patients	%	No. of Patients	%	No. of Patients	%
Sex																
Male	92	55	89	53	66	55	57	50	44	60	30	49	22	47	27	52
Female	76	45	80	47	54	45	56	50	29	40	31	51	25	53	25	48
Age, years																
Median	60		62		60		59		59		59		61		60	
Range	30-82		24-82		30-82		24-82		36-82		24-74		30-75		41-82	
< 65	109	65	96	57	77	64	70	62	46	63	39	64	31	66	31	60
≥ 65	59	35	73	43	43	36	43	38	27	37	22	36	16	34	21	40
Region																
Eastern Europe	93	55	97	57	71	59	70	62	45	62	38	62	26	55	32	62
Western Europe	49	29	45	27	49	41	43	38	28	38	23	38	21	45	20	38
Southern Europe	25	15	27	16	0		0		0		0		0		0	
ROW	1	1	0		0		0		0		0		0		0	
ECOG PS at baseline																
0	75	45	65	39	50	42	34	30	27	37	19	31	23	49	15	29
1	76	45	89	53	56	47	70	62	37	51	37	61	19	40	33	63
2	17	10	15	9	14	12	9	8	9	12	5	8	5	11	4	8
Disease duration, months																
Median, CRC	2.2		2.1		2.1		2.2		2.0		2.1		2.1		2.3	
Q1-Q3	1.3-17.6		1.2-9.8		1.3-14.4		1.4-10.7		1.3-15.0		1.2-9.4		1.2-7.8		1.6-10.7	
Median, mCRC	1.5		1.4		1.6		1.4		1.6		1.3		1.4		1.6	
Q1-Q3	1.1-2.3		0.9-2.3		1.1-2.3		1.0-2.4		1.2-2.2		0.9-2.4		1.1-2.3		1.0-2.4	
Tumor site																
Colon	89	53	92	54	66	55	61	54	41	56	31	51	25	53	30	58
Rectum	79	47	75	44	54	45	51	45	32	44	30	49	22	47	21	40
Colon + rectum	0		2	1	0		1	1	0		0		0		1	2
Metastatic sites																
Liver	146	87	149	88	108	90	101	89	65	89	54	89	43	91	47	90
Lung	65	39	64	38	44	37	45	40	27	37	16	26	17	36	29	56
Bone	4	2	9	5	3	3	4	4	3	4	1	2	0		3	6
Lymph node	45	27	41	24	32	27	25	22	19	26	15	25	13	28	10	19
Other	27	16	25	15	17	14	17	15	12	16	9	15	5	11	8	15
Organs with metastases																
0	1*	0.6	0		0		0		0		0		0		0	
1	69	41	74	44	52	43	50	44	31	42	32	52	21	45	18	35
2	63	38	60	36	41	34	38	34	24	33	17	28	17	36	21	40
3	18	11	22	13	15	13	16	14	12	16	9	15	3	6	7	13
≥ 4	17	10	13	8	12	10	9	8	6	8	3	5	6	13	6	12
Prior therapy†																
Surgery	152	91	137	81	108	90	100	88	67	92	50	82	41	87	50	96
Adjuvant chemotherapy	36	21	21	12	19	16	14	12	12	16	8	13	7	15	6	12
Radiotherapy	23	14	18	11	13	11	13	12	9	12	5	8	4	9	8	15

Abbreviations: ITT, intention to treat; FOLFOX-4, oxaliplatin, leucovorin, and fluorouracil; ROW, rest of the world; ECOG PS, Eastern Cooperative Oncology Group performance status; CRC, colorectal cancer; mCRC, metastatic colorectal cancer; Q1-Q3, interquartile range.

*Patient enrolled in violation of protocol.

†A patient may have received more than one prior therapy.

to 14 spanning locked nucleic acid oligomer to suppress amplification of the wild-type sequence.²⁹ Post-PCR hybridization and melting curve analysis using fluorescently tagged oligonucleotides incorporated in the original PCR reaction permitted the identification and discrimination of distinct *KRAS* codon 12 and 13 missense mutations.

Safety Analysis

The safety population comprised all patients who received at least one dose of study treatment. All adverse events (AEs) reported with onset on or after the first day of study medication and captured up to 6 weeks after the end of the last administration of study treatment were coded using the Medical Dictionary for Regulatory Activities version 8.1 and summarized by worst severity per patient according to the National Cancer Institute Common Toxicity Criteria version 2.0.

RESULTS

Patient Demographics

Three hundred forty-four patients were randomly assigned (three of them in error). Of the eligible patients, 337 received study

treatment at 79 centers. Study accrual lasted from July 2005 until March 2006, with 169 patients receiving cetuximab plus FOLFOX-4 and 168 patients receiving FOLFOX-4 alone (Fig 1). Baseline demographic and disease characteristics were generally well balanced between the treatment groups (Table 1). DNA suitable for *KRAS* mutation analysis was extracted from the tumor samples of 233 patients, 113 and 120 of whom had received cetuximab plus FOLFOX-4 and FOLFOX-4 alone, respectively. These patients comprised the *KRAS* population. In general, the population with tissue available for *KRAS* analysis ($n = 233$) was considered to be representative of the overall ITT population ($n = 337$) in relation to demographic parameters (Table 1).

Treatment Compliance

The median duration of cetuximab treatment was 24 weeks, with 84% of patients having a relative dose-intensity (RDI) of $\geq 80\%$. Similar numbers of patients in both arms had RDIs of $\geq 80\%$ for oxaliplatin (75% and 80% of patients receiving cetuximab plus

Table 2. Efficacy Data in the ITT and *KRAS* Populations

Characteristic	ITT Population (n = 337)				<i>KRAS</i> Population (n = 233)				<i>KRAS</i> Wild Type (n = 134)				<i>KRAS</i> Mutant (n = 99)			
	FOLFOX-4 (n = 168)		Cetuximab + FOLFOX-4 (n = 169)		FOLFOX-4 (n = 120)		Cetuximab + FOLFOX-4 (n = 113)		FOLFOX-4 (n = 73)		Cetuximab + FOLFOX-4 (n = 61)		FOLFOX-4 (n = 47)		Cetuximab + FOLFOX-4 (n = 52)	
	No. of Patients	%	No. of Patients	%	No. of Patients	%	No. of Patients	%	No. of Patients	%	No. of Patients	%	No. of Patients	%	No. of Patients	%
Best overall response*																
Complete response	1	0.6	2	1	3	3	2	2	1	1	2	3	2	4	0	
Partial response	59	35	75	44	47	39	52	46	26	36	35	57	21	45	17	33
Stable disease	76	45	67	40	47	39	46	41	30	41	19	31	17	36	27	52
Progressive disease	21	13	18	11	17	14	10	9	12	16	3	5	5	11	7	13
Not assessable	11	7	7	4	6	5	3	3	4	5	2	3	2	4	1	2
Best overall response rate†, %																
All patients	36		46		42		48		37		61		49		33	
95% CI	29-44		38-53		33-51		38-57		26-49		47-73		34-64		20-47	
Odds ratio		1.516				1.312				2.544				0.507		
95% CI		0.975-2.355				0.779-2.208				1.238-5.227				0.223-1.150		
P (stratified CMH test)		.064				.307				.011				.106		
Disease control rate‡, %	81		85		81		89		78		92		85		85	
95% CI	74-87		79-90		73-87		81-94		67-87		82-97		72-94		72-93	
PFS time, months																
Median	7.2		7.2		7.2		7.3		7.2		7.7		8.6		5.5	
95% CI	6.0-7.8		5.6-7.7		6.0-7.9		5.6-8.1		5.6-7.4		7.1-12.0		6.5-9.5		4.0-7.4	
Hazard ratio		0.931				0.928				0.570				1.830		
95% CI		0.705-1.230				0.665-1.295				0.358-0.907				1.095-3.056		
P (log-rank test)		.6170				.6609				.0163				.0192		
PFS rates, %																
3 months	85		83		82		86		78		93		87		78	
95% CI	79-90		78-89		75-89		80-93		69-88		87-100		77-97		67-90	
6 months	59		53		60		54		54		66		69		39	
95% CI	51-68		45-61		50-69		44-64		42-66		53-79		54-83		25-54	
9 months	34		34		33		35		27		47		42		20	
95% CI	26-43		26-43		23-43		25-45		15-39		33-62		24-61		7-33	
12 months	12		24		13		20		13		30		14		6	
95% CI	3-20		15-33		4-23		8-32		2-24		10-49		0-30		0-16	

NOTE. $P < 0.05$ for bold values.

Abbreviations: ITT, intention to treat; FOLFOX-4, oxaliplatin, leucovorin, and fluorouracil; CMH, Cochran-Mantel-Haenszel; PFS, progression-free survival.

*As assessed by independent review. Best overall response on the ITT population was based on data available at the a priori fixed time of the confirmatory analysis.

†Complete response + partial response.

‡Complete response + partial response + stable disease.

FOLFOX-4 and FOLFOX-4, respectively) and FU (67% and 70% of patients, respectively). Reductions and delays in cetuximab dosing were primarily because of skin reactions, and delays in chemotherapy dosing were because of hematologic, GI, or neurologic reactions. In the *KRAS* population, comparable levels of exposure were reached; 86% of patients who received cetuximab had a RDI \geq 80%. RDIs of \geq 80% were also reported for 73% and 81% of patients receiving oxaliplatin and for 65% and 72% of patients receiving FU in the cetuximab plus FOLFOX-4 and FOLFOX-4 arms, respectively.

Efficacy

Efficacy data are summarized in Table 2. The best confirmed overall response rate in the cetuximab plus FOLFOX-4 group was 46%, and in the FOLFOX-4-alone group, this value was 36%. The comparison between the two treatment groups resulted in a common ECOG PS-adjusted OR of 1.52 (95% CI, 0.98 to 2.36), indicating an increase in the chance of a response in the cetuximab plus FOLFOX-4 arm by 52%. This increase was not statistically significant at the 5% level (ECOG PS-adjusted CMH test, $P = .064$). A slightly greater treatment effect was observed in the group of participants ($n = 153$ v 152) with ECOG PS 0 to 1 (OR = 1.65; 95% CI, 1.04 to 2.60; CMH test, $P = .032$). Regarding the secondary efficacy end points, although a marked benefit in relation to PFS time was not detected (Table 2; Fig 2A), the addition of cetuximab to FOLFOX-4 was associated with an approximate doubling of the R0 resection rate from 2.4% (four of 168 patients) for FOLFOX-4 alone to 4.7% (eight of 169 patients) for cetuximab plus FOLFOX-4. The median duration of response was also longer in the cetuximab group (9.0 v 5.7 months).

KRAS mutations in codons 12/13 (hereinafter called *KRAS* mutations) were detected in the tumor tissue of 99 of 233 (42%) patients, 52 of whom received cetuximab plus FOLFOX-4 and 47 of whom received FOLFOX-4 alone (Table 2). No mutations were detected in the tumors of 134 patients (58%; hereinafter called *KRAS* wild-type tumors), 61 of whom received cetuximab plus

FOLFOX-4 and 73 of whom received FOLFOX-4 alone. In patients whose tumors were scored as *KRAS* wild type, the addition of cetuximab to FOLFOX-4 was associated with a 2.54-fold increased chance for response compared with FOLFOX-4 alone (61% v 37%; OR = 2.54; 95% CI, 1.24 to 5.23; ECOG PS-adjusted CMH test, $P = .011$). Conversely, for patients with tumor *KRAS* mutations, the response rate for cetuximab plus FOLFOX-4 was lower, compared with FOLFOX-4 alone (33% v 49%; OR = 0.51; 95% CI, 0.22 to 1.15; ECOG PS-adjusted CMH test, $P = .106$). The R0 resection rate was more than doubled in patients with *KRAS* wild-type tumors who received cetuximab plus FOLFOX-4 (six of 61 patients; 9.8%) compared with those who received FOLFOX-4 alone (three of 73 patients; 4.1%). In contrast, in patients whose tumors carried *KRAS* mutations, R0 resection rates were similar in both treatment arms (one in 52 and one in 47 patients, respectively; 1.9% and 2.1%). However, due to the small patient numbers, these data have to be interpreted with caution.

The risk of disease progression (Figs 2A and 2B) was similar for both the ITT (cetuximab plus FOLFOX-4 v FOLFOX-4 alone; hazard ratio [HR] = 0.93; log-rank test, $P = .6170$) and the *KRAS* population (HR = 0.93; log-rank test, $P = .6609$), adding additional evidence consistent with the representability of the *KRAS* population. A marked risk reduction for progression was evident between the study arms when comparing patients according to whether *KRAS* mutations were detected in their tumor DNA. Specifically, as summarized in Table 2 and Figure 3, the addition of cetuximab to FOLFOX-4 was associated with a 43% reduction in the risk of progression in patients with *KRAS* wild-type tumors (Fig 3C; HR = 0.57; log-rank test, $P = .0163$). In those patients receiving cetuximab plus FOLFOX-4, the risk of disease progression was lower for those whose tumors were wild type for *KRAS*, compared with those whose tumors carried mutations (Fig 3A; HR = 0.45; log-rank test, $P = .0009$). However, in patients whose tumors carried mutations of *KRAS*, the risk of disease progression was found to be lower for those receiving FOLFOX-4 alone compared with those receiving cetuximab plus FOLFOX-4 (Fig 3D;

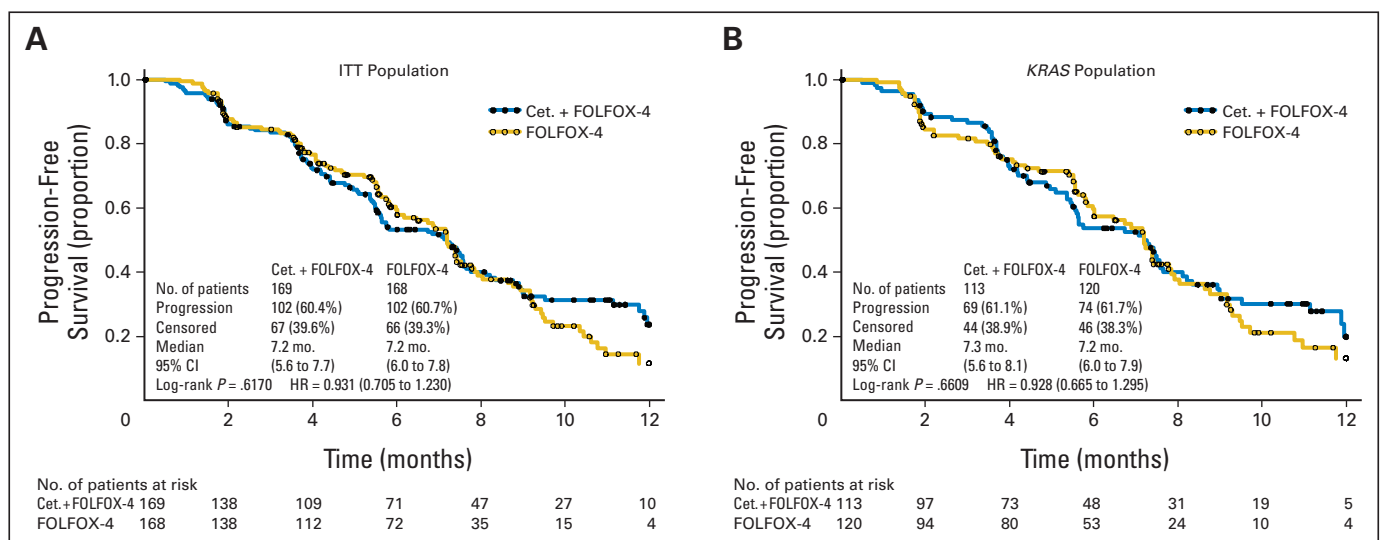


Fig 2. Kaplan and Meier plots for progression-free survival time for patients in each study arm in (A) the intention to treat (ITT) population and (B) the *KRAS* population. Cet., cetuximab; FOLFOX-4, oxaliplatin, leucovorin, and fluorouracil; mo., months.

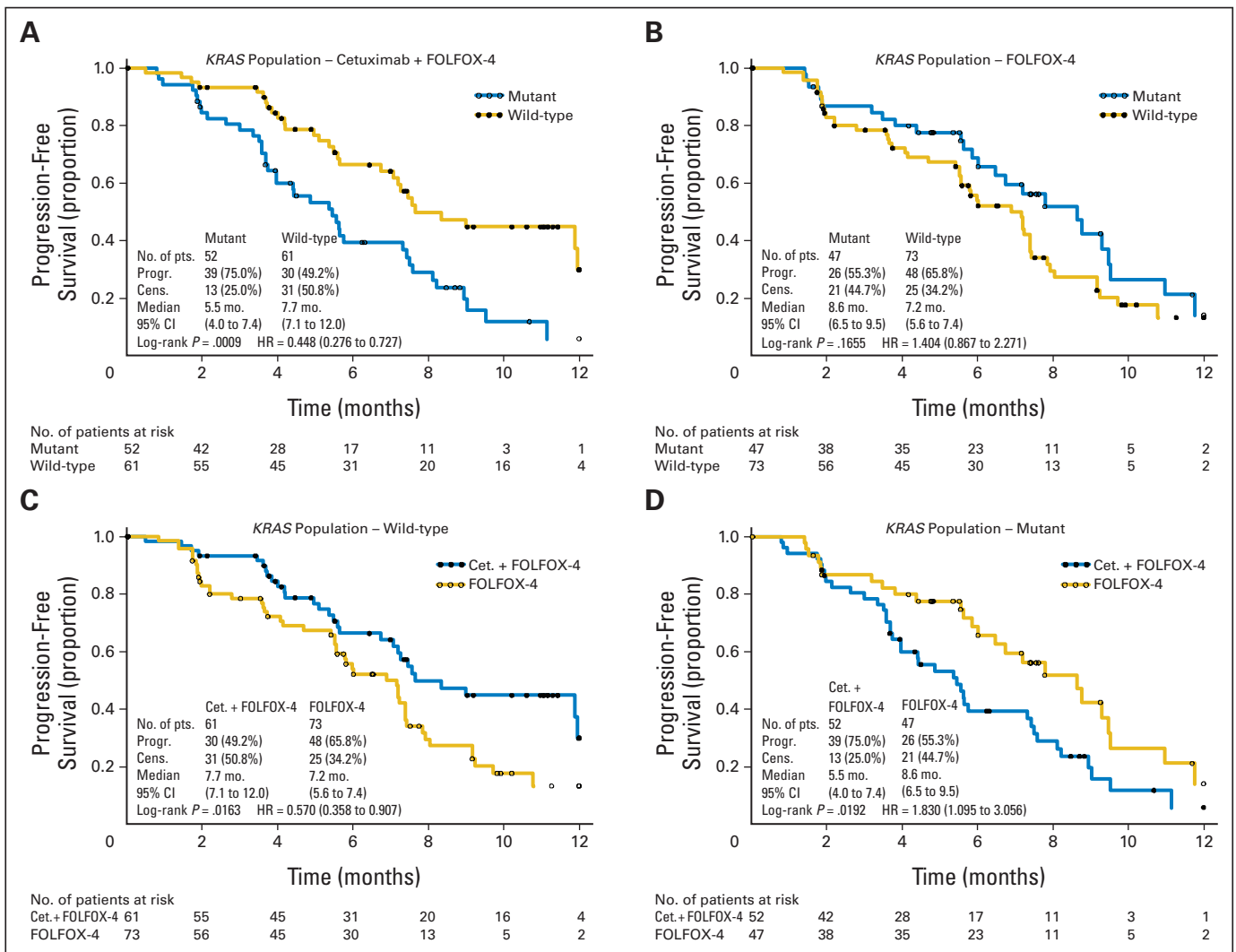


Fig 3. Kaplan and Meier plots for progression-free survival time according to tumor *KRAS* mutation status in patients (pts) who received (A) cetuximab (Cet.) plus oxaliplatin, leucovorin, and fluorouracil (FOLFOX-4) or (B) FOLFOX-4 alone and according to treatment arm for pts whose tumors were (C) wild type or (D) mutant for *KRAS*. Progr., progression; Cens., censored; HR, hazard ratio.

HR = 1.83; log-rank test, *P* = .0192). For patients receiving FOLFOX-4 alone, a trend for reduced risk of disease progression associated with tumor *KRAS* mutation was also apparent (Fig 3B; HR = 1.40; log-rank test, *P* = .1655).

Safety

The most common AEs at any grade were skin and subcutaneous tissue disorders (90%) and GI disorders (78%) in the cetuximab plus FOLFOX-4 group, and GI disorders (73%) and myelotoxicity (71%) in the FOLFOX-4-alone group. The most common grade 3/4 AEs were neutropenia (30% v 34%), rash (11% v 0.6%), and diarrhea (8% v 7%) respectively, for the cetuximab plus FOLFOX-4 versus the FOLFOX-4-alone group (Table 3).

In the cetuximab plus FOLFOX-4 group, cetuximab was discontinued in 39 patients (23%), chemotherapy was discontinued in 51 patients (30%), and both were discontinued in 16 patients (9%) due to AEs. Chemotherapy was discontinued in 42 patients (25%) in the FOLFOX-4-alone group due to AEs. The most com-

mon reasons for cetuximab discontinuation were hypersensitivity reactions (4%) and rash (4%), in accordance with guidelines pre-specified in the protocol, and for chemotherapy discontinuation in the cetuximab plus FOLFOX-4 group, neutropenia (4%) and neuropathy (3%). In the FOLFOX-4-alone group, peripheral sensory neuropathy (5%), neuropathy and paresthesia (4%), and neutropenia (2%) were the most common reasons for treatment discontinuation.

At the time of analysis, 111 patients had died; 60 patients (35%) in the cetuximab plus FOLFOX-4 group and 51 patients (30%) in the FOLFOX-4-alone group, mainly due to PD (94 patients). No deaths were related to cetuximab.

DISCUSSION

The rationale for testing cetuximab in combination with FOLFOX-4 rested on preclinical data suggesting that cetuximab improved the antitumor activity of oxaliplatin and also on promising clinical phase II study data.^{16,30} The confirmed best overall response rate

Table 3. Relevant Grade 3 or 4 Adverse Events Occurring in $\geq 3\%$ of Patients in Either Group

Grade 3/4 Adverse Event	FOLFOX-4 (n = 168)		Cetuximab + FOLFOX-4 (n = 170)	
	No. of Patients	%	No. of Patients	%
Any grade 3/4 event	117	70	129	76
Neutropenia	57	34	51	30
Rash*	1	0.6	19	11
Diarrhea*	12	7	14	8
Leukopenia	10	6	12	7
Thrombocytopenia	4	2	7	4
Fatigue*	5	3	7	4
Palmar-plantar erythrodysesthesia*	1	0.6	7	4
Peripheral sensory neuropathy*	12	7	7	4
Anemia*	4	2	6	4
Composite categories				
Skin reactions*†	1	0.6	30	18
Infusion-related reactions‡	3	2	8	5

Abbreviation: FOLFOX-4, oxaliplatin, leucovorin, and fluorouracil.

*No grade 4 reactions.

†The special adverse event category skin reactions included the following *Medical Dictionary for Regulatory Activities 8.1* terms: acne, acne pustular, cellulitis, dermatitis acneiform, dry skin, erysipelas, erythema, face edema, folliculitis, growth of eyelashes, hair growth abnormal, hypertrichosis, nail bed infection, nail bed inflammation, nail disorder, nail infection, paronychia, pruritus, rash, rash erythematous, rash follicular, rash generalized, rash macular, rash maculopapular, rash popular, rash pruritic, rash pustular, skin exfoliation, skin hyperpigmentation, skin necrosis, staphylococcal scalded skin syndrome, telangiectasia, wound necrosis, xerosis.

‡The special adverse event category infusion-related reactions included the following *Medical Dictionary for Regulatory Activities 8.1* terms: acute respiratory failure, apnea, asthma, bronchial obstruction, bronchospasm, cyanosis, dyspnea, dyspnea at rest, dyspnea exacerbated, dyspnea exertional, hypoxia, orthopnea, respiratory distress, respiratory failure, chills, hyperpyrexia, pyrexia, acute myocardial infarction, angina pectoris, blood pressure decreased, cardiac failure, cardiopulmonary failure, clonus, convulsion, epilepsy, hypotension, infusion related reaction, loss of consciousness, myocardial infarction, myocardial ischemia, shock, sudden death and syncope, occurring on the first day of treatment; and anaphylactic reaction, anaphylactic shock, anaphylactoid reaction, anaphylactoid shock, drug hypersensitivity and hypersensitivity, occurring at any time during treatment.

for the ITT population, as assessed by blinded independent review, was increased by 10 percentage points by the addition of cetuximab to FOLFOX-4, compared with FOLFOX-4 alone (46% *v* 36%). Although this difference showed a clinically relevant and strong trend, a statistically significant increase in the odds for a response with the addition of cetuximab to FOLFOX-4 could not be established ($P = .064$).

Although there was no apparent difference in PFS time between the ITT population treatment arms, when retrospective efficacy analyses were carried out in the *KRAS* population according to *KRAS* mutation status, striking differences were apparent in relation to tumor response and the risk of disease progression. Patients whose tumors were wild type for *KRAS* had a clinically relevant increase in the chance of response (61% *v* 37%; OR = 2.54; $P = .011$) and decrease in the risk of disease progression (HR = 0.57; $P = .016$) in the cetuximab plus FOLFOX-4 arm compared with those in the FOLFOX-4-alone arm. Somewhat unexpected was the observation that patients whose tumors carried a mutation in *KRAS* were more likely to derive a clinical benefit if treated with FOLFOX-4 alone rather than cetuximab plus FOLFOX-4 (Fig 3D). Similarly,

considering patients receiving FOLFOX-4 alone, there was a trend for improved progression-free survival time in patients whose tumors carried a *KRAS* mutation (Fig 3B).

It remains to be seen whether the apparently better outcome of patients whose tumors carried *KRAS* mutations who were treated with FOLFOX-4 alone compared with those treated with cetuximab plus FOLFOX-4 was a chance finding, or whether it might have a biologic explanation. Given that a comparable effect was not observed in equivalent patient groups in the CRYSTAL study when cetuximab was added to FOLFIRI,²³ the type of chemotherapy (platinum based compared with nonplatinum based) may be a factor in any possible interaction. It is interesting to note in this context that the subgroup of genotyped patients in the randomized phase III CAIRO2 study whose tumors had a mutation in the *KRAS* gene ($n = 196$) who received capecitabine/oxaliplatin plus bevacizumab ($n = 103$) had significantly better PFS than those who received the same regimen plus cetuximab ($n = 93$).³¹

The OPUS and CAIRO2 studies therefore raise the possibility that in relation to tumors with mutations in the *KRAS* gene, the addition of EGFR-targeted agents to oxaliplatin-based chemotherapy may impair the efficacy of a cytotoxic component of the combined regimen. In addition, a specific interaction between bevacizumab and cetuximab in relation to the treatment of tumors with *KRAS* mutations cannot be excluded. Experimental studies are therefore warranted to explore such hypothetical treatment effects in *KRAS* mutant mCRCs with a view to devising novel clinical strategies to allow extension of the benefit of anti-EGFR therapy to patients whose tumors have *KRAS* mutations.

In line with recent findings from a phase I dose-escalation study of cetuximab with FOLFIRI,³² and the first-line data from the phase III CRYSTAL study,²³ the results of the current analysis confirm published data from previously treated mCRC, regarding restriction of the activity of cetuximab to patients whose tumors are wild type for *KRAS*.^{19-22,24} The *KRAS* data also emphasize the fact that the higher clinical benefit associated with cetuximab essentially may be masked, or at the least underestimated, when considering overall populations of unselected patients in randomized studies, as illustrated by the nonsignificant results in response rate and the comparable risk of disease progression in the ITT population.

The combination of cetuximab and FOLFOX-4 was generally well tolerated, and there was no evidence to suggest that cetuximab increased the frequency or severity of the known toxicities of oxaliplatin and FU or vice versa. In addition, the incidence and severity of skin reactions, infusion-related reactions, and mucositis were consistent with the known safety profile of cetuximab.

In conclusion, this study demonstrated that to a clinically meaningful extent, the addition of cetuximab to FOLFOX-4 as first-line treatment for mCRC increased the overall response rate and reduced the risk of disease progression in patients whose tumors were wild type for *KRAS*, compared with FOLFOX-4 alone. These data confirm that *KRAS* mutation status is a powerful predictive factor in relation to the efficacy of cetuximab treatment, and are a clear demonstration of the potential and increasing importance of tumor biomarker analysis as a component of treatment selection in the clinic.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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Correlation of efficacy to KRAS status (wt vs. mut)

in patients (pts) with metastatic colorectal cancer
(mCRC), treated with weekly (q1w) and q2w
schedules of cetuximab combined with FOLFIRI.

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Appendix

Participating Centers

Investigators at 87 centers in 13 countries enrolled patients onto the initial epidermal growth factor receptor screening phase of the study. Austria: Richard Greil (St Johannis Spital-Landeskrankenhaus, Salzburg), Heinz Ludwig (Wilhelminenspital der Stadt Wien, Wien), Dieter Lutz (A. ö. KH der Elisabethinen Linz, Linz), Hellmut Samonigg (Medizinische Universitätsklinik, Graz), Johannes Schüller (KH der Stadt Wien Rudolfstiftung, Wien), Ewald Wöll (Krankenhaus St Vinzenz Zams, Zams); Belgium: Filip Baert (Heilig Hartziekenhuis, Roeselare), Alain Bols (AZ Sint-Jan, Brugge), Geert D'Haens (Imeldaziekenhuis, Bonheiden), Jos Janssens (Sint-Elisabethziekenhuis, Turnhout), Eric Joosens (AZ Middelheim, Antwerpen), Joseph Kerger (UCL Mont Godinne, Yvoir), Jeroen Mebis (Virga Jesse Ziekenhuis, Hasselt), Marc Peeters (UZ Gent, Gent), Eric Van Cutsem (UZ Gasthuisberg, Leuven); France: Dominique Béchade (Hôpital d'Instruction des Armées du Val de Grâce, Paris), Christophe Borg (Hôpital Jean Minjos, Besancon Cedex), Marian Gil Delgado (Groupe Hospitalier Pitié-Salpêtrière, Paris Cedex 13), Jean Louis Misset (Hôpital Saint Louis, Paris), Eric Raymond (Hôpital Beaujon, Clichy), Jean-Philippe Wagner (Clinique Sainte Anne, Strasbourg), Marc Ychou (C.R.L.C. 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Methods: KRAS Mutation Analysis

Pathology. Formaldehyde-fixed, paraffin-embedded (FFPE) tumor biopsy samples, as paraffin blocks or as 3- to 5- μ m paraffin sections mounted on glass slides, were sent to a central facility (Merck Serono, Darmstadt, Germany) for *KRAS* mutation analysis. Serial sections were cut from each block for pathologic analysis (3 μ m) and DNA extraction (10 μ m, stored at -80°C pending isolation). A pathologist confirmed the

presence of invasive tumor cells in the tissue through examination of hematoxylin and eosin–stained sections. If invasive tumor cells could not be confirmed in a particular sample, the biopsy was excluded from further analysis.

DNA extraction. For FFPE tissue blocks, DNA was extracted from three to six 10- μ m sections (depending on biopsy size). If tumor samples were available only as sections mounted on glass slides, the complete sample was scraped off and transferred into a 1.5-mL tube using a fresh scalpel for each slide, to avoid cross contamination.

Paraffin was dissolved by adding 800 μ L xylene to each sample followed by gentle mixing. Subsequently, 400 μ L ethanol (100% v/v) was added to each tube. The mixture was centrifuged for 2 minutes at 13,000 rpm in a microcentrifuge and the supernatant was carefully removed and discarded. If any remaining paraffin was visible, the xylene and ethanol extraction was repeated. The pellet was washed with 1,000 μ L of ethanol (100% v/v) and the tubes were centrifuged for 2 minutes at 13,000 rpm. The supernatant was again carefully removed and the pellet containing the DNA dried for 10 minutes at 55°C. Genomic DNA was isolated from the dried pellet using the DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The concentration of each extracted DNA sample was determined by measuring the absorption at 260 nm using a Nanodrop UV/VIS spectrometer (Thermo Fisher Scientific, Wilmington, DE). Purity was assessed by determination of the 260/280 nm ratio. The DNA quality was further assessed in a *KRAS*-independent quantitative polymerase chain reaction (qPCR) amplification. Genomic DNA from cell lines was also isolated using the DNeasy Blood & Tissue Kit, according to the manufacturer's instructions and quantified and quality controlled as for the FFPE material.

Methods: *KRAS* Mutation Detection by Locked Nucleic Acid–Mediated qPCR Clamping and Melting Curve Analysis

Locked nucleic acid–mediated qPCR clamping. PCR reactions were performed on a LightCycler 2.0 system, using a *KRAS* mutation detection–specific program with additional melting curve analysis, in a one-step qPCR amplification and detection run using the LightMix k-ras Gly12 assay (TIB MOLBIOL, Berlin, Germany). The methodology was adapted from Chen et al.²⁸ PCR reactions incorporated: a *KRAS*-specific primer pair (*KRAS* forward, *KRAS* reverse), which amplified a 164 base pair genomic fragment from *KRAS* exon 2; two hybridization sensor probes covering the mutation hotspot region around codons 12 and 13; and an anchor probe (Anchor FL), which was 3'-end labeled with fluorescein. The sensor probes were 5'-end labeled with LC Red dye and 3'-end phosphorylated to prevent extension. Amplification of *KRAS* wild-type DNA in the PCR reaction was inhibited by temperature stable binding of a *KRAS* wild-type specific locked nucleic acid sequence (2'-4' bridged ribose backbone).

The mutation analysis was performed in duplicate for each tumor sample. The PCR reaction was performed in a total volume of 20 μ L containing: 7.4 μ L PCR-grade water, 1.6 μ L 25 mM MgCl₂, 4.0 μ L of reagent/primer mix (parameter specific reagents containing both PCR primers, one anchor, two sensor-probes, and the LNA), 2.0 μ L of qPCR Mastermix (Roche Diagnostics, Penzberg, Germany), and 5 μ L of the genomic DNA solution (50 ng). PCR reactions incorporating a 1:10 mixture of DNA (1 ng) derived from cell lines with defined *KRAS* mutations and DNA (10 ng) from cell lines with wild-type *KRAS* were included as positive controls in each run. PCR reactions with 10 ng of DNA derived from cells with wild-type *KRAS* were performed as negative controls in each run. The qPCR conditions comprised a preincubation of 600 seconds at 95°C followed by 45 cycles of 95°C for 10 seconds, 58°C for 5 seconds, and 72°C for 12 seconds.

Melting curve analysis. After the qPCR run was complete, a melting curve analysis was performed by measuring the fluorescence of the samples with increasing temperature. Initially, the PCR products were denatured at 95°C. Subsequent cooling of the reaction mix allowed the anchor probe and the mutation-specific sensor probes to anneal to the amplified genomic *KRAS* sequence, resulting in increased fluorescence due to fluorescence resonance energy transfer between annealed anchor and sensor probe dyes. The melting curve was assessed by heating the sample from 40°C to 85°C, with continuous monitoring of fluorescence in the 640/530-nm channel. At a certain temperature, depending on the identity of the *KRAS* mutation, binding of the sensor probe to the DNA strand was disrupted. This resulted in spatial separation of the fluorescence resonance energy transfer partners, and as a consequence, in a decrease of fluorescence. The change of fluorescence intensity was converted to a melting peak (T_m) by plotting the negative derivative of fluorescent signal corresponding to the temperature ($-dF/dT$). The melting curve analysis program therefore comprised a preincubation of 20 seconds at 95°C, followed by 20 seconds at 58°C, followed by 10 seconds at 40°C, followed by heating to 85°C at 0.3°C/second. Different individual *KRAS* mutations displayed different characteristic T_m values. Each could be identified by comparing the T_m of the experimental sample with the T_m values of a panel of positive controls.