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ORIGINAL REPORT

Fluorouracil, Leucovorin, and Irinotecan Plus Cetuximab Treatment and *RAS* Mutations in Colorectal Cancer

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ABSTRACT

Purpose
The phase III CRYSTAL study demonstrated that addition of cetuximab to fluorouracil, leucovorin, and irinotecan (FOLFIRI) significantly improved overall survival, progression-free survival, and objective response in the first-line treatment of patients with *KRAS* codon 12/13 (exon 2) wild-type metastatic colorectal cancer (mCRC). Outcome was reassessed in subgroups defined by extended *RAS* mutation testing.

Patients and Methods
Existing DNA samples from *KRAS* exon 2 wild-type tumors from CRYSTAL study patients were reanalyzed for other *RAS* mutations in four additional *KRAS* codons (exons 3 and 4) and six *NRAS* codons (exons 2, 3, and 4) using beads, emulsion, amplification, and magnetics technology. No tissue microdissection was performed. A ≥ 5% mutant allele cutoff was used to call mutations.

Results
Mutation status was evaluable in 430 (64.6%) of 666 patients with *KRAS* exon 2 wild-type tumors. Other *RAS* mutations were detected in 63 (14.7%) of 430 patients. In those with *RAS* wild-type tumors, a significant benefit across all efficacy end points was associated with the addition of cetuximab to FOLFIRI. In patients with other *RAS* tumor mutations, no difference in efficacy outcomes between treatment groups was seen. The safety profile in *RAS* subgroups was similar and in line with expectations.

Conclusion
In the first-line treatment of mCRC, patients with *RAS* wild-type tumors derived a significant benefit from the addition of cetuximab to FOLFIRI; patients with *RAS* tumor mutations did not. Molecular testing of tumors for all activating *RAS* mutations is essential before considering anti–epidermal growth factor receptor therapy, thereby allowing the further tailoring of cetuximab administration to maximize patient benefit.

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INTRODUCTION

The randomized phase III CRYSTAL (Cetuximab Combined With Irinotecan in First-Line Therapy for Metastatic Colorectal Cancer) study showed that the addition of cetuximab to infusional fluorouracil, leucovorin, and irinotecan (FOLFIRI) significantly improved overall survival (OS) time, progression-free survival (PFS) time, and objective response rate in the first-line treatment of patients with *KRAS* codon 12 and 13 (hereinafter exon 2) wild-type metastatic colorectal cancer (mCRC). No cetuximab efficacy benefit was apparent in the subgroup of patients whose tumors carried such exon 2 mutations.[1,2]

In the same setting, the randomized phase II OPUS (Oxaliplatin and Cetuximab in First-Line Treatment of Metastatic Colorectal Cancer) study demonstrated that the addition of cetuximab to another standard first-line regimen—fluorouracil, leucovorin, and oxaliplatin (FOLFOX4)—significantly improved objective response rate and PFS time in patients with *KRAS* exon 2 wild-type tumors. However, it was reported that for patients with *KRAS* exon 2 tumor mutations, the addition of cetuximab to FOLFOX4 resulted in worse outcome compared with FOLFOX4 alone.[3,4] Similar findings were also reported from the PRIME (Panitumumab Randomized Trial in Combination With Chemotherapy for Metastatic Colorectal Cancer to Determine Efficacy) study for another epidermal growth factor receptor (EGFR) antibody, panitumumab, which improved outcome in patients when combined with FOLFOX4 in the first-line treatment of *KRAS* exon 2 wild-type mCRC. As for cetuximab, a negative effect

was apparent when panitumumab was combined with FOLFOX4 in patients with *KRAS* exon 2 mutations.[5]

Activating missense mutations of *KRAS* at codons other than 12 or 13 have been identified in a wide range of tumor types, including CRC.[6] Similar somatic tumor mutations have also been detected at corresponding loci within the *NRAS* gene. A retrospective analysis of the PRIME study demonstrated that a subgroup of 17.4% of patients with *KRAS* exon 2 wild-type mCRC had tumor mutations at another *RAS* locus (*KRAS* codons 61, 117, and 146; *NRAS* codons 12, 13, and 61). Such other *RAS* mutations were associated negatively with outcome in patients receiving FOLFOX4 plus panitumumab.[7] Exploratory analysis further suggested that *RAS* codon 59 mutations might also be negative biomarkers for panitumumab efficacy.

The primary objective of our post hoc investigation was to assess the treatment effect of FOLFIRI plus cetuximab compared with FOLFIRI alone in patients with tumors carrying predefined mutations at *RAS* loci other than *KRAS* codon 12 or 13 (other *RAS* mutations). Also assessed was the treatment effect in patients with evaluable tumors wild type at all *RAS* loci.

PATIENTS AND METHODS

Study Design and Treatment

The phase III CRYSTAL study compared 14-day cycles of FOLFIRI plus weekly cetuximab with FOLFIRI alone as first-line treatment for patients with EGFR-expressing mCRC. Treatment was continued until disease progression, unacceptable toxicity, or withdrawal of consent. The primary end point was PFS, as determined by independent review. The clinical study was approved by the independent ethics committee of each trial center and was carried out in accordance with the Declaration of Helsinki. Eligibility criteria have been described previously.[1] All patients provided informed consent before inclusion. A previous retrospective subgroup analysis investigated the association of tumor *KRAS* exon 2 mutation status and treatment outcome.[2]

RAS *Mutation Testing*

Extended *RAS* mutation testing was performed on DNA samples extracted previously from formalin-fixed paraffin-embedded tumor tissue sections from CRYSTAL study patients and which had been scored in earlier investigations as wild type at codons 12 and 13 of *KRAS*.[2] Before DNA extraction, stained slides were reviewed by a pathologist to estimate overall neoplastic cell content; no exclusion criteria were applied. The estimated fraction of neoplastic cells was between 5% and 60% for most samples. Because the polymerase chain reaction clamping and melting curve technique used in the original testing was a highly sensitive method designed to enrich for mutant over wild-type sequences,[1,8] macro- or microdissection of tissue sections was not carried out. In the application of the 5% cutoff in our analysis, the estimated fraction of neoplastic cells was not taken into consideration. Ploidy status, which was not assessed, was also not taken into consideration.

The highly sensitive beads, emulsion, amplification, and magnetics (BEAM) technology was selected for the *RAS* mutation analysis to take into account the source and nature of the tumor DNA available (Data Supplement).[9] This approach can detect and enumerate mutant versus wild-type DNA sequences at ratios down to 1:10,000 (0.01%).[10,11] *RAS* testing was carried out by a contract research organization (Sysmex Inostics, Hamburg, Germany).

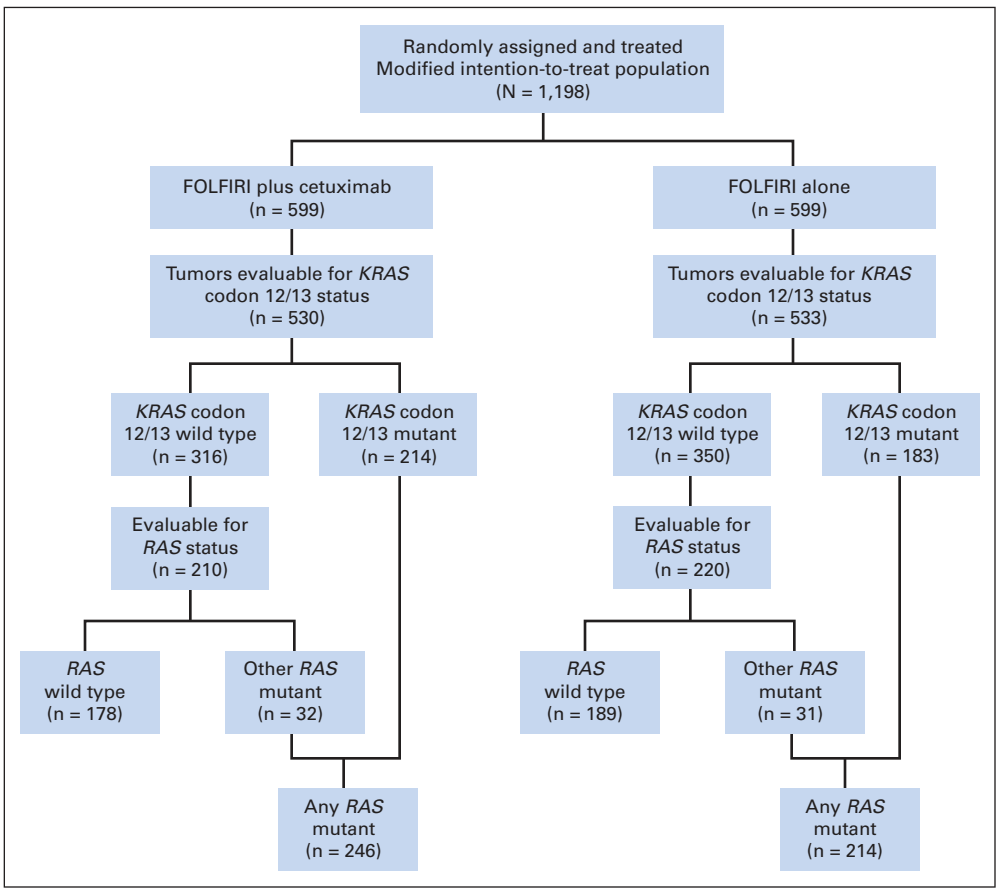

Fig 1. Study profile. FOLFIRI, fluorouracil, leucovorin, and irinotecan.

Table 1. Baseline Demographic and Clinical Characteristics of *KRAS* Exon 2 Wild-Type Subgroup of *KRAS* Population and *RAS* Subpopulations

	KRAS Exon 2 Wild Type																			
	Overall (N = 666)				*RAS* Evaluable (n = 430)				*RAS* Wild Type (n = 367)				Other *RAS* Mutations (n = 63)				Any *RAS* Mutation (n = 460)			
	FOLFIRI + Cetuximab (n = 316)		FOLFIRI (n = 350)		FOLFIRI + Cetuximab (n = 210)		FOLFIRI (n = 220)		FOLFIRI + Cetuximab (n = 178)		FOLFIRI (n = 189)		FOLFIRI + Cetuximab (n = 32)		FOLFIRI (n = 31)		FOLFIRI + Cetuximab (n = 246)		FOLFIRI (n = 214)	
Characteristic	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Sex																				
Male	196	62.0	211	60.3	130	61.9	137	62.3	109	61.2	120	63.5	21	65.6	17	54.8	152	61.8	123	57.5
Female	120	38.0	139	39.7	80	38.1	83	37.7	69	38.8	69	36.5	11	34.4	14	45.2	94	38.2	91	42.5
Age, years																				
Median	61.0		59.0		60.0		59.0		60.0		59.0		61.5		60.0		62.0		63.0	
Range	24.0-79.0		19.0-84.0		24.0-79.0		19.0-82.0		24.0-79.0		19.0-82.0		28.0-75.0		30.0-78.0		22.0-80.0		30.0-83.0	
Region																				
Western Europe	145	45.9	158	45.1	103	49.0	107	48.6	86	48.3	95	50.3	17	53.1	12	38.7	110	44.7	96	44.9
Eastern Europe	115	36.4	123	35.1	75	35.7	70	31.8	63	35.4	56	29.6	12	37.5	14	45.2	88	35.8	80	37.4
Outside Europe	56	17.7	69	19.7	32	15.2	43	19.5	29	16.3	38	20.1	3	9.4	5	16.1	48	19.5	38	17.8
ECOG PS																				
0	183	57.9	200	57.1	116	55.2	131	59.5	97	54.5	114	60.3	19	59.4	17	54.8	143	58.1	108	50.5
1	120	38.0	136	38.9	85	40.5	80	36.4	76	42.7	68	36.0	9	28.1	12	38.7	92	37.4	101	47.2
2	13	4.1	14	4.0	9	4.3	9	4.1	5	2.8	7	3.7	4	12.5	2	6.5	11	4.5	5	2.3
Duration of mCRC, months																				
Mean	3.4		2.5		3.4		2.6		3.6		2.5		2.3		3.2		3.1		2.2	
SD	8.3		4.9		8.6		5.2		9.3		5.1		1.5		5.9		5.4		3.0	
Median	1.6		1.5		1.6		1.6		1.6		1.6		2.0		1.6		1.7		1.6	
Range	0-92		0-66		0-92		0-66		0-92		0-66		0-7		0-32		0-43		0-32	
Prior adjuvant chemotherapy	80	25.3	73	20.9	48	22.9	44	20.0	40	22.5	42	22.2	8	25.0	2	6.5	40	16.3	28	13.1
No. of metastatic sites																				
≤ 2	277	87.7	295	84.3	183	87.1	187	85.0	157	88.2	161	85.2	26	81.3	26	83.9	209	85.0	179	83.6
> 2	33	10.4	49	14.0	23	11.0	30	13.6	17	9.6	25	13.2	6	18.8	5	16.1	35	14.2	33	15.4
Missing	6	1.9	6	1.7	4	1.9	3	1.4	4	2.2	3	1.6	0	0.0	0	0.0	2	0.8	2	0.9
Metastases confined to liver	68	21.5	72	20.6	47	22.4	52	23.6	43	24.2	46	24.3	4	12.5	6	19.4	49	19.9	56	26.2
Longest diameter of index lesion																				
≤ Median	165	52.2	184	52.6	112	53.3	119	54.1	92	51.7	101	53.4	20	62.5	18	58.1	126	51.2	106	49.5
> Median	150	47.5	164	46.9	98	46.7	99	45.0	86	48.3	87	46.0	12	37.5	12	38.7	119	48.4	105	49.1
Missing	1	0.3	2	0.6	0	0.0	2	0.9	0	0.0	1	0.5	0	0.0	1	3.2	1	0.4	3	1.4
Tumor site																				
Colon	183	57.9	204	58.3	124	59.0	132	60.0	106	59.6	117	61.9	18	56.3	15	48.4	153	62.2	131	61.2
Rectum	127	40.2	140	40.0	81	38.6	85	38.6	68	38.2	70	37.0	13	40.6	15	48.4	86	35.0	79	36.9
Colon and rectum	6	1.9	6	1.7	5	2.4	3	1.4	4	2.2	2	1.1	1	3.1	1	3.2	6	2.4	4	1.9
Missing	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	0.4	0	0.0
Tumor localization*																				
Left sided	246	77.8	267	76.3	164	78.1	165	75.0	142	79.8	138	73.0	22	68.8	27	87.1	164	66.7	152	71.0
Right sided	64	20.3	80	22.9	42	20.0	55	25.0	33	18.5	51	27.0	9	28.1	4	12.9	76	30.9	62	29.0
Left and right sided	6	1.9	3	0.9	4	1.9	0	0.0	3	1.7	0	0.0	1	3.1	0	0.0	5	2.0	0	0.0
Missing	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	0.4	0	0.0
Alkaline phosphatase, U/L																				
≥ 300	30	9.5	42	12.0	25	11.9	18	8.2	22	12.4	17	9.0	3	9.4	1	3.2	33	13.4	24	11.2
< 300	272	86.1	295	84.3	177	84.3	191	86.8	150	84.3	163	86.2	27	84.4	28	90.3	203	82.5	175	81.8
Missing	14	4.4	13	3.7	8	3.8	11	5.0	6	3.4	9	4.8	2	6.3	2	6.5	10	4.1	15	7.0
Leucocytes, per μL																				
≤ 10,000	258	81.6	284	81.1	176	83.8	181	82.3	150	84.3	154	81.5	26	81.3	27	87.1	200	81.3	160	74.8
> 10,000	48	15.2	58	16.6	27	12.9	32	14.5	22	12.4	29	15.3	5	15.6	3	9.7	38	15.4	50	23.4
Missing	10	3.2	8	2.3	7	3.3	7	3.2	6	3.4	6	3.2	1	3.1	1	3.2	8	3.3	4	1.9
Lactate dehydrogenase																				
> ULN	138	43.7	150	42.9	97	46.2	87	39.5	84	47.2	73	38.6	13	40.6	14	45.2	107	43.5	99	46.3
≤ ULN	144	45.6	161	46.0	90	42.9	110	50.0	74	41.6	96	50.8	16	50.0	14	45.2	116	47.2	87	40.7
Missing	34	10.8	39	11.1	23	11.0	23	10.5	20	11.2	20	10.6	3	9.4	3	9.7	23	9.3	28	13.1

Abbreviations: ECOG PS, Eastern Cooperative Oncology Group performance status; FOLFIRI, fluorouracil, leucovorin, and irinotecan; mCRC, metastatic colorectal cancer; SD, standard deviation; ULN, upper normal limit.

*Right sided: appendix, cecum, ascending colon, hepatic flexure, and transverse colon. Left sided: splenic flexure, descending colon, sigmoid colon, and rectum.

Table 2. Efficacy Data in *KRAS* Exon 2 Wild-Type Subgroup of *KRAS* Population and *RAS* Subpopulations

	KRAS Exon 2 Wild Type																			
	Overall (N = 666)				*RAS* Evaluable (n = 430)				*RAS* Wild Type (n = 367)				Other *RAS* Mutation (n = 63)				Any *RAS* Mutation (n = 460)			
	FOLFIRI + Cetuximab (n = 316)		FOLFIRI (n = 350)		FOLFIRI + Cetuximab (n = 210)		FOLFIRI (n = 220)		FOLFIRI + Cetuximab (n = 178)		FOLFIRI (n = 189)		FOLFIRI + Cetuximab (n = 32)		FOLFIRI (n = 31)		FOLFIRI + Cetuximab (n = 246)		FOLFIRI (n = 214)	
Efficacy	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
OS, months																				
Median	23.5		20.0		26.1		20.2		28.4		20.2		18.2		20.7		16.4		17.7	
95% CI	21.2 to 26.3		17.4 to 21.7		23.3 to 29.0		17.3 to 23.4		24.7 to 31.6		17.0 to 24.5		11.3 to 24.8		12.2 to 25.8		14.9 to 18.4		15.4 to 19.6	
HR	0.80				0.75				0.69				1.22				1.05			
95% CI	0.67 to 0.95				0.60 to 0.93				0.54 to 0.88				0.69 to 2.16				0.86 to 1.28			
*P**	.0093				.0080				.0024				.50				.64			
PFS, months																				
Median	9.9		8.4		11.3		7.7		11.4		8.4		7.2		6.9		7.4		7.5	
95% CI	9.0 to 11.3		7.4 to 9.2		9.5 to 13.3		7.3 to 9.2		10.0 to 14.6		7.4 to 9.4		4.6 to 9.6		5.4 to 7.2		6.4 to 8.0		7.2 to 8.5	
HR	0.70				0.58				0.56				0.81				1.10			
95% CI	0.56 to 0.87				0.44 to 0.77				0.41 to 0.76				0.39 to 1.67				0.85 to 1.42			
*P**	.0012				< .001				< .001				.56				.47			
Best overall response†																				
Complete	3	0.9	0	0.0	2	1.0	0	0.0	2	1.1	0	0.0	0	0.0	0	0.0	0	0.0	2	0.9
Partial	178	56.3	139	39.7	127	60.5	84	38.2	116	65.2	73	38.6	11	34.4	11	35.5	78	31.7	75	35.0
Stable disease	100	31.6	162	46.3	63	30.0	107	48.6	48	27.0	90	47.6	15	46.9	17	54.8	116	47.2	101	47.2
Progressive disease	19	6.0	31	8.9	10	4.8	19	8.6	7	3.9	17	9.0	3	9.4	2	6.5	27	11.0	22	10.3
Not evaluable	16	5.1	18	5.1	8	3.8	10	4.5	5	2.8	9	4.8	3	9.4	1	3.2	25	10.2	14	6.5
Objective response rate, %	57.3		39.7		61.4		38.2		66.3		38.6		34.4		35.5		31.7		36.0	
95% CI	51.6 to 62.8		34.6 to 45.1		54.5 to 68.1		31.7 to 45.0		58.8 to 73.2		31.7 to 46.0		18.6 to 53.2		19.2 to 54.6		25.9 to 37.9		29.6 to 42.8	
OR	2.07				2.64				3.11				1.02				0.85			
95% CI	1.52 to 2.83				1.78 to 3.92				2.03 to 4.78				0.33 to 3.15				0.58 to 1.25			
P‡	< .001				< .001				< .001				.97				.40			

NOTE. Analyses were stratified according to Eastern Cooperative Oncology Group performance status (0 or 1 *v* 2) and region (Western Europe *v* Eastern Europe *v* rest of world).
Abbreviations: FOLFIRI, fluorouracil, leucovorin, and irinotecan; HR, hazards ratio; OR, odds ratio; OS, overall survival; PFS, progression-free survival.
*Log-rank test.
†As assessed by independent review.
‡Cochran-Mantel-Haenszel test.

The presence of 26 specific mutations reported in the Catalogue of Somatic Mutations in Cancer and/or Cancer Genome Atlas databases within *KRAS* exons 3 (codons 59 and 61) and 4 (codons 117 and 146) and *NRAS* exons 2 (codons 12 and 13), 3 (codons 59 and 61), and 4 (codons 117 and 146) was investigated (Data Supplement). Samples were not reassessed for the presence of *KRAS* exon 2 (codons 12 and 13) mutations.

Statistical Analysis

For each evaluable sample, the fraction of mutant *RAS* alleles was calculated by dividing the number of mutant beads by the total number of beads with polymerase chain reaction product (sum of mutant, wild-type, and mutant/wild-type beads). Broadly in line with the sensitivity of other approaches that might be used clinically to assign *RAS* mutation status, and after an initial evaluation of the impact of using alternative cutoff values, tumors were scored as *RAS* mutant if the sum of the individual percentages of mutant sequences over total amplified sequences for the analyzed loci was $\geq 5\%$, regardless of whether all loci were evaluable for mutation status. Tumors were scored as *RAS* wild type only if all 26 mutation assays were evaluable and the summed prevalence of mutations across all loci was $< 5\%$.

The primary objective of our post hoc analysis was to investigate the treatment effect of FOLFIRI plus cetuximab compared with FOLFIRI alone in patients with tumor *RAS* mutations other than *KRAS* exon 2 (other *RAS*). This extended analysis was limited to those CRYSTAL study patients for whom an evaluable tumor DNA sample was available. Outcome for patients with tumors wild type at all tested *RAS* loci was also investigated. Treatment outcome in patients with any *RAS* mutation, comprising those with either previously identified *KRAS* exon 2 mutations (not reassessed in our study) or other *RAS* mutations, was also analyzed. Outcome in patients wild type for both *RAS* and *BRAF* (V600E; as previously defined[2]) was considered. The treatment effect in different other *RAS*-mutant populations, as defined according to a range of diagnostic cutoffs from 0.1% to 20%, was visualized using forest plots of hazard ratios (HRs) and odds ratios (ORs).

HRs and ORs were calculated for FOLFIRI plus cetuximab versus FOLFIRI alone. For OS and PFS, HRs and 95% CIs for treatment comparisons were calculated using univariable Cox proportional hazards models. Median survival times were estimated using the Kaplan-Meier method[12] (product-limit estimates), and *P* values were calculated using log-rank tests. For objective response, treatment groups were compared using Cochran-Mantel-Haenszel tests. All analyses, apart from those for different cutoff values as presented in the forest plots, were stratified according to Eastern Cooperative Oncology Group performance status and region, as assigned through the interactive voice response system at random assignment.

RESULTS

Patients and Samples

Of the 666 CRYSTAL study patients with tumors previously typed as *KRAS* exon 2 wild type, *RAS* tumor mutation status was evaluable in 430 (64.6%; Fig 1). Samples from the remaining 236 patients were not evaluable, either because of insufficient residual DNA or because of the failure of ≥ 1 mutation assay. Using a 5% mutant/wild-type cutoff, other *RAS* mutations were scored in 63 (14.7%) of 430 patients. Those with other *RAS* mutations were grouped with patients previously classified as having tumors with *KRAS* exon 2 mutations (n = 397) to comprise a combined population of patients with any *RAS* mutations (n = 460). Using the less stringent cutoff of $\geq 0.1\%$ mutant/wild-type sequences, other *RAS* mutations were scored in 86 (20.0%) of 430 patients. The most common site of other *RAS* mutations was within *KRAS* exon 4 (Data Supplement).

Of 422 *RAS*/*BRAF* evaluable tumors, 46 (10.9%) were known to carry *BRAF* mutations, all but one of which were scored as *RAS* wild type; 315 (74.6%) of 422 tumors were wild type for both *RAS* and *BRAF*.

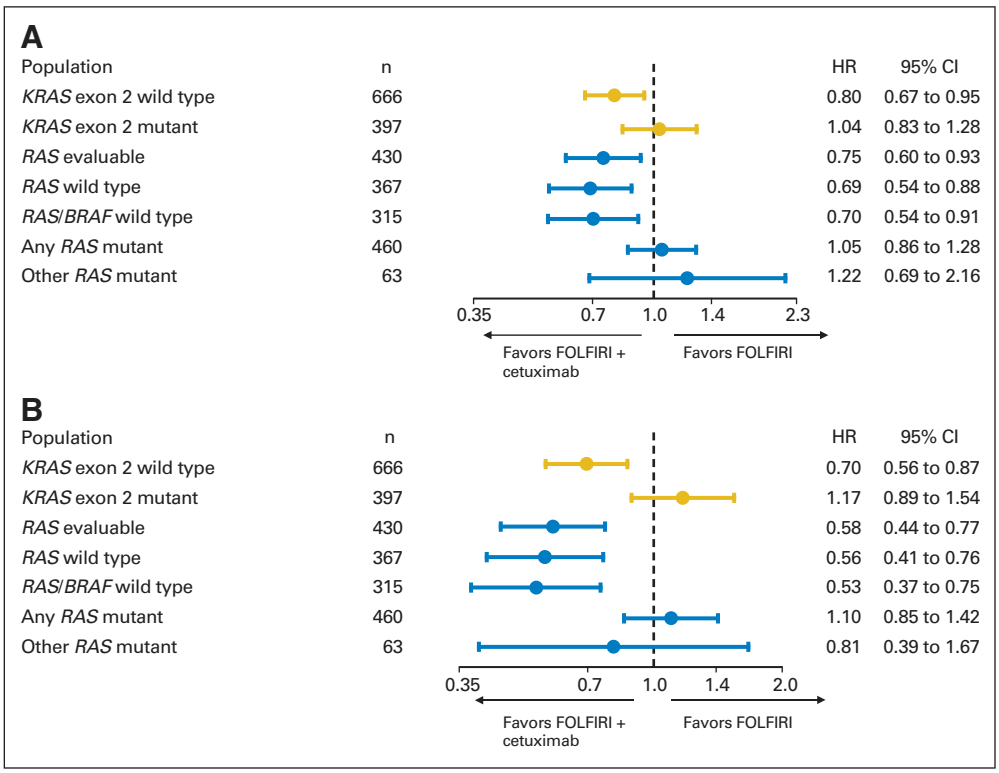

Fig 2. Hazard ratios (HRs) for (A) overall and (B) progression-free survival according to tumor *KRAS* exon 2 and *RAS* mutation status. FOLFIRI, fluorouracil, leucovorin, and irinotecan.

Comparability of Evaluable Populations

Baseline characteristics of the *RAS*-evaluable population and *RAS* wild-type and other *RAS*-mutant subgroups were broadly similar to those of the *KRAS* exon 2 wild-type subgroup of the *KRAS* population (Table 1). However, and although balanced between treatment groups, notably fewer patients with a tumor *RAS* mutation (68 [14.8%] of 460) had received prior adjuvant chemotherapy compared with those with *RAS* wild-type tumors (82 [22.3%] of 367).

In relation to efficacy, there was a trend for better outcome for the FOLFIRI-plus-cetuximab group compared with the FOLFIRI-alone group in the *RAS*-evaluable population compared with the *KRAS* exon 2 wild-type population, with the difference most pronounced in relation to PFS and objective response (Table 2; Fig 2). Given the similarity in baseline characteristics between the *KRAS* exon 2 wild-type population and the *RAS*-evaluable subset, there was no obvious selection bias that might have explained the increased cetuximab benefit in the *RAS*-evaluable population.

Efficacy According to RAS *Mutation Status*

A clear and significant benefit associated with the addition of cetuximab to FOLFIRI was apparent in relation to OS, PFS, and objective response in patients with *RAS* wild-type tumors (n = 367; efficacy outcome summarized in Table 2 and Figs 2 to 4). The HR for OS time and OR for objective response rate were more favorable toward FOLFIRI plus cetuximab in the *RAS* wild-type population than in the *RAS*-evaluable population. Setting aside the possible issue of selection bias, results for the *RAS* wild-type/*BRAF* wild-type population were similar to those of the *RAS* wild-type population (Data Supplement). For patients with tumors harboring other *RAS* mutations (n = 63), no clear cetuximab treatment benefit was apparent. Efficacy outcomes were also investigated for the group of patients with any *RAS* mutation (n = 460). No clear difference in efficacy outcome between treatment groups was apparent in this population.

BEAMing analysis allowed for the detection of tumor *RAS* mutations at low prevalence. Using a more sensitive threshold of 0.1% to call mutations, 23 (5.3%) of 430 tumors classified as *RAS* wild type when using a 5% cutoff were instead scored as *RAS* mutant. Efficacy outcomes were reassessed in *RAS* wild-type and *RAS*-mutant populations, as defined according to this 0.1% cutoff (Data Supplement). As for the 5% cutoff analysis, HRs and ORs did

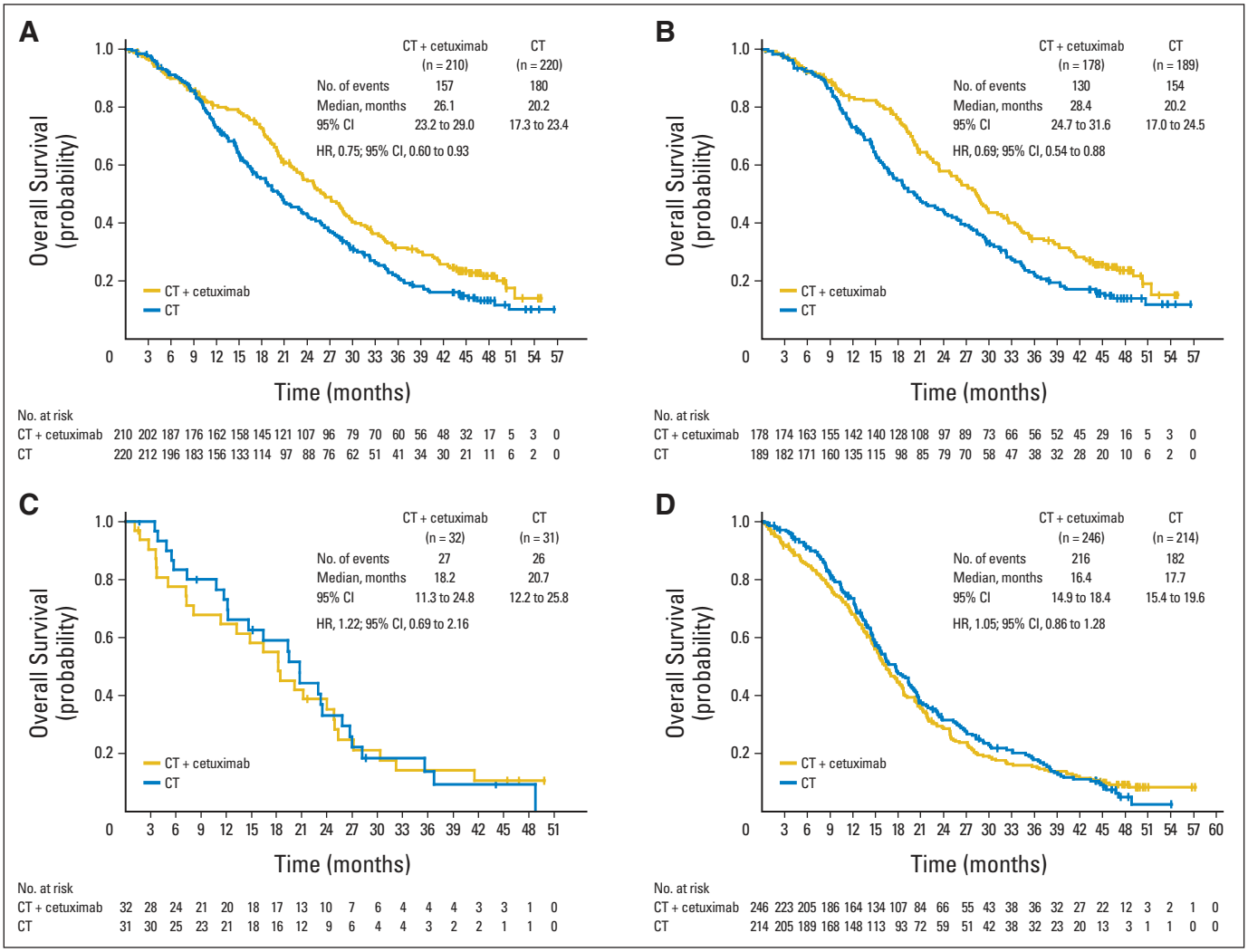

Fig 3. Overall survival according to treatment group in *RAS* populations. (A) *KRAS* codon 12 or 13 wild type, evaluable for other *RAS* mutations. (B) *RAS* wild type (all loci). (C) *KRAS* codon 12 or 13 wild type; other *RAS* mutations. (D) *RAS* mutation (any locus). CT, chemotherapy; HR, hazard ratio.

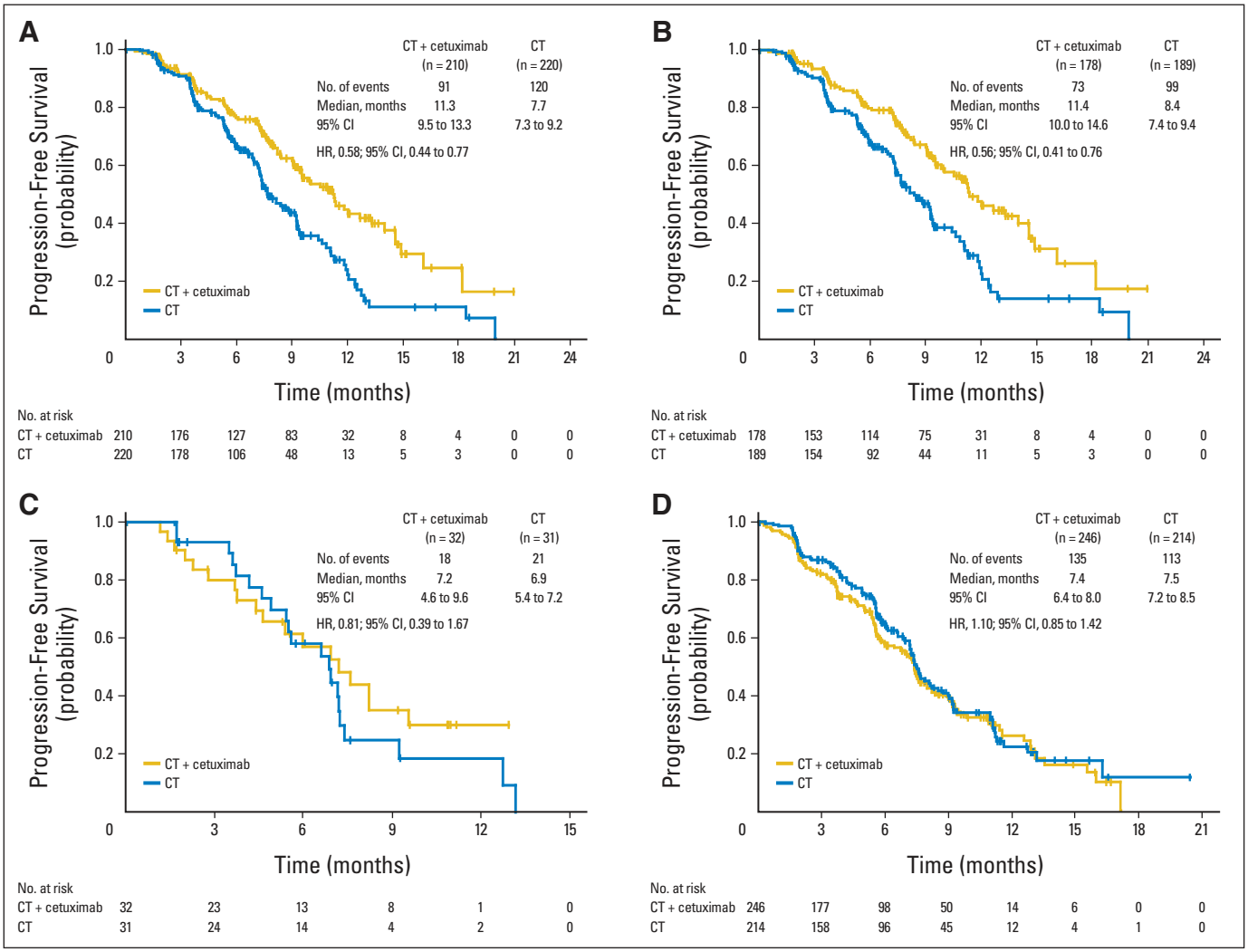

Fig 4. Progression-free survival according to treatment group in *RAS* populations. (A) *KRAS* codon 12 or 13 wild type, evaluable for other *RAS* mutations. (B) *RAS* wild type (all loci). (C) *KRAS* codon 12 or 13 wild type; other *RAS* mutations. (D) *RAS* mutation (any locus). CT, chemotherapy; HR, hazard ratio.

not suggest a positive or negative effect on efficacy associated with the addition of cetuximab to FOLFIRI in patients with tumor *RAS* mutations.

Treatment outcome in other *RAS*-mutant populations defined according to cutoff values of 20%, 10%, 5%, 1%, and 0.1% were visualized using forest plots of HRs for OS and PFS times and ORs for objective response rates (Data Supplement). These plots suggested that there was a relationship between the fraction of *RAS*-mutated neoplastic cells in a tumor and the strength of EGFR-targeted therapy resistance and that patients with other tumor *RAS* mutation signals between 0.1% and $< 5\%$ may have benefited from the addition of cetuximab to FOLFIRI.

Safety

The overall incidence of adverse events according to treatment group was broadly similar across the *KRAS* and *RAS* subgroups (Table 3). In addition, the incidence of commonly reported adverse events in each treatment group was also generally similar across these populations and in line with expectations.

DISCUSSION

The aim of our post hoc analysis of the CRYSTAL study was to investigate the impact of *RAS* mutations other than *KRAS* codon 12 or 13 in relation to treatment effects. In the evaluable patients with other tumor *RAS* mutations, there was no clear evidence that the addition of cetuximab to FOLFIRI modified efficacy outcome. However, given the relatively small number of patients in this group, no definitive conclusions on the negative predictive value of other *RAS* mutations can be drawn. In the combined group of patients with any *RAS* mutation (*KRAS* exon 2 or other *RAS*), there was no indication that the addition of cetuximab to FOLFIRI either improved or worsened outcome. The absence of a negative effect in the *RAS*-mutant subgroup when cetuximab was combined with FOLFIRI in our study contrasts strongly with the data from the OPUS[13] and PRIME[7] *RAS* investigations, which suggested worse outcome in the *RAS*-mutant subgroups associated with the addition of cetuximab (PFS: HR, 1.54; 95% CI, 1.04 to 2.29) or panitumumab (PFS: HR, 1.31; 95% CI, 1.07

Table 3. Most Common Grade 3 to 4 AEs in *RAS* Subgroups of Safety Population

	KRAS Exon 2 Wild Type																			
	Overall (N = 666)*				*RAS* Evaluable (n = 430)†				*RAS* Wild Type (n = 367)†				Other *RAS* Mutation (n = 63)†				Any *RAS* Mutation (n = 460)†			
	FOLFIRI + Cetuximab (n = 316)		FOLFIRI (n = 350)		FOLFIRI + Cetuximab (n = 210)		FOLFIRI (n = 220)		FOLFIRI + Cetuximab (n = 178)		FOLFIRI (n = 189)		FOLFIRI + Cetuximab (n = 32)		FOLFIRI (n = 31)		FOLFIRI + Cetuximab (n = 246)		FOLFIRI (n = 214)	
AE	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Any	257	81.1	211	60.3	169	80.5	131	59.5	144	80.9	110	58.2	25	78.1	21	67.7	189	76.8	133	62.1
MedDRA preferred term																				
Neutropenia	97	30.6	83	23.7	64	30.5	46	20.9	55	30.9	38	20.1	9	28.1	8	25.8	62	25.2	58	27.1
Diarrhea	52	16.4	35	10.0	29	13.8	20	9.1	26	14.6	18	9.5	3	9.4	2	6.5	30	12.2	22	10.3
Rash	28	8.8	0	0.0	19	9.0	0	0.0	16	9.0	0	0.0	3	9.4	0	0.0	19	7.7	0	0.0
Leucopenia	25	7.9	17	4.9	15	7.1	10	4.5	15	8.4	7	3.7	0	0.0	3	9.7	12	4.9	13	6.1
Fatigue	14	4.4	20	5.7	12	5.7	11	5.0	12	6.7	9	4.8	0	0.0	2	6.5	16	6.5	5	2.3
Deep vein thrombosis	16	5.0	2	0.6	11	5.2	2	0.9	11	6.2	1	0.5	0	0.0	1	3.2	2	0.8	9	4.2
Dermatitis acneiform	16	5.0	0	0.0	11	5.2	0	0.0	9	5.1	0	0.0	2	6.3	0	0.0	14	5.7	0	0.0
Composite categories*																				
Skin reactions																				
Any	67	21.1	1	0.3	46	21.9	1	0.5	37	20.8	1	0.5	9	28.1	0	0.0	49	19.9	0	0.0
Acne-like rash	52	16.4	0	0.0	36	17.1	0	0.0	30	16.9	0	0.0	6	18.8	0	0.0	41	16.7	0	0.0
Infusion-related reactions	5	1.6	0	0.0	4	1.9	0	0.0	4	2.2	0	0.0	0	0.0	0	0.0	7	2.8	0	0.0

NOTE. Reported at frequency of ≥ 5% in either treatment group of *KRAS* exon 2 wild-type subgroup of safety population and according to composite categories of special interest.
Abbreviations: AE, adverse event; FOLFIRI, fluorouracil, leucovorin, and irinotecan; MedDRA, Medical Dictionary for Regulatory Activities.
*According to MedDRA (version 10.0).
†According to MedDRA (version 12.0; except for composite categories).

to 1.60 and OS: HR, 1.25; 95% CI, 1.02 to 1.55), respectively, to FOLFOX4. However, our data are in line with findings in the second-line setting, which showed that there was no negative effect of combining panitumumab with FOLFIRI for patients with *KRAS* exon 2–mutant or other *RAS*-mutant tumors.[14,15] Furthermore, on the basis of the findings from these studies, current clinical guidelines recommend that only patients with *RAS* wild-type tumors should be treated with cetuximab or panitumumab in combination with FOLFIRI or FOLFOX chemotherapy.[16]

In patients with *RAS* wild-type tumors, a clear cetuximab benefit was seen across efficacy end points. However, it should be noted in this context that an increased cetuximab benefit was apparent in the *RAS*-evaluable subgroup, compared with the overall *KRAS* exon 2 wild-type population, regardless of *RAS* status. Despite the similarity of baseline characteristics, selection bias in relation to the *RAS*-evaluable subgroup cannot be excluded.

The method selected to screen for *RAS* mutations was the BEAMing technique.[9] Although this approach can detect *KRAS* exon 2 mutations at a level of 0.01% mutant to wild-type sequences,[17] a higher threshold of ≥ 5% was used in our *RAS* study. This is broadly in line with the sensitivity of other techniques such as next-generation sequencing, pyrosequencing, and dideoxy nucleotide sequencing, which may be used clinically to determine *RAS* mutation status. Because the fraction of neoplastic cells and ploidy status were not taken into account, the percentage of mutated cells cannot be estimated.

Using this cutoff, *RAS* mutations were scored in 14.7% of evaluable CRYSTAL study patients with *KRAS* exon 2 wild-type tumors. This frequency is similar to those reported in other first-line mCRC studies, which used pyrosequencing or dideoxy sequencing/WAVE analysis.[7,18,19] In common with these studies and also the parallel *RAS* analysis of the OPUS study,[13] the most common location of *RAS* mutations outside of *KRAS* exon 2 in CRYSTAL study patients was *KRAS* exon 4. Also of interest in relation to the mutation profile was that *NRAS* exon 4 tumor mutations, which were scored in four CRYSTAL study patients when using the 5% cutoff and in 12 patients when using the 0.1% cutoff, had not to our knowledge been reported previously in the tumors of patients receiving first-line treatment for mCRC. *NRAS* exon 4 tumor mutations were also seen in the OPUS study *RAS* analysis,[13] in which an identical BEAMing mutation detection approach was used; again, the incidence was higher when 0.1% rather than 5% was used as a cutoff. It may be that such mutations tend to be of low prevalence and, although present, may not have been detected by other screening technologies.

Because the significance of low-prevalence *KRAS* or *RAS* mutations in relation to the effectiveness of EGFR antibody therapy in mCRC is not clear,[20-23] we also explored treatment outcome in *RAS* subgroups defined according to a threshold of 0.1% mutant to wild-type sequences. The effect of using the lower cutoff (higher sensitivity in relation to mutation identification) was to move 23 patients previously classified as *RAS* wild type to the mutant group. This resulted in essentially no change in the HRs or ORs for FOLFIRI plus cetuximab over FOLFIRI alone in the revised *RAS* wild-type population and marginally better outcome for the FOLFIRI-plus-cetuximab group in the revised other *RAS*-mutant population. These data are therefore consistent with patients with low-prevalence mutations (between 0.1% and < 5% mutant to wild type) deriving a treatment benefit from the addition of cetuximab to FOLFIRI. This conclusion is in line with the findings from a retrospective study of 95 patients with mCRC

who had received EGFR antibody therapy, which indicated that the PFS of patients with tumors with low-prevalence *KRAS* mutations (< 5%) was comparable to that of patients with *KRAS* wild-type tumors.[21] Using higher cutoff levels resulted in too few patients for meaningful interpretations, but our forest plots suggest that the higher the fraction of mutated cells, the stronger the resistance to EGFR antibody treatment. This would be in line with the hypothesis that acquired resistance to such agents may result, at least in part, from the outgrowth of small numbers of cells with existing *RAS* mutations.[20] The CRYSTAL study data are therefore consistent with < 5% mutant sequences being a potentially appropriate cutoff value for determining eligibility for FOLFIRI plus cetuximab as first-line treatment. Nevertheless, more data and more precise measurements of the fraction of mutated neoplastic cells, as well as an accurate definition of the association between the fraction of mutated cells and resistance to EGFR monoclonal antibodies, are needed to define the optimal cutoff for the clinical setting. This would require large collaborative studies using common standardized methodologies for tumor processing and *RAS* mutation detection.

In summary, our study supports the use of FOLFIRI plus cetuximab in patients with *RAS* wild-type tumors and, on the basis of a lack of observed benefit, suggests the exclusion of patients with other *RAS* mutations. Reserving such first-line treatment for a *RAS* wild-type population allows the definition of a subgroup more likely to benefit from the addition of cetuximab to FOLFIRI. Molecular testing of tumors for all activating mutations in *KRAS* and *NRAS* before considering anti-EGFR therapy is therefore essential in selecting the most effective treatment for patients with mCRC.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Disclosures provided by the authors are available with this article at www.jco.org.

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Fluorouracil, Leucovorin, and Irinotecan Plus Cetuximab Treatment and *RAS* Mutations in Colorectal Cancer

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