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ORIGINAL REPORT

Analysis of Potential Predictive Markers of Cetuximab Benefit in BMS099, a Phase III Study of Cetuximab and First-Line Taxane/Carboplatin in Advanced Non–Small-Cell Lung Cancer

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See accompanying editorial on page 903 and article on page 911

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ABSTRACT

Purpose
The anti–epidermal growth factor receptor (EGFR) antibody cetuximab is efficacious in multiple tumor types. Patient selection with markers predictive of benefit may enhance its therapeutic index. This retrospective, correlative analysis of the phase III trial BMS099 of cetuximab in advanced non–small-cell lung cancer (NSCLC) was conducted to identify molecular markers for the selection of patients most likely to benefit from cetuximab.

Methods
In BMS099, 676 chemotherapy-naïve patients with stage IIIB (pleural effusion) or stage IV NSCLC of any histology or EGFR expression status were randomly assigned to taxane/carboplatin (T/C) with or without cetuximab. Biomarkers analyzed included *K-Ras* and *EGFR* mutations by direct sequencing, EGFR protein expression by immunohistochemistry (IHC), and *EGFR* gene copy number by fluorescent in situ hybridization (FISH). Relationships between biomarker status and progression-free survival (PFS), overall survival (OS), and overall response rate (ORR) were assessed by log-rank tests per treatment arm for treatment-specific effects and across the total evaluable population.

Results
Tumor samples were available from 225 randomly assigned patients. *K-Ras* mutations were found in 17% of evaluable patients (35 of 202 patients), *EGFR* mutations were found in 10% (17 of 166 patients), EGFR positivity by IHC was found in 89% (131 of 148 patients), and FISH positivity was found in 52% (54 of 104 patients). No significant associations were found between biomarker status and PFS, OS, and ORR in the treatment-specific analyses.

Conclusion
In contrast with colorectal cancer, and within the limitations of the data set, efficacy parameters did not appear to correlate with *K-Ras* mutation status or with any of the EGFR-related biomarkers evaluated. Additional exploratory analyses are essential to identify predictive markers and to optimize patient selection for cetuximab therapy in NSCLC.

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INTRODUCTION

Cetuximab (Erbitux; Bristol-Myers Squibb, Princeton, NJ; ImClone Systems, New York, NY) is an anti–epidermal growth factor receptor (EGFR) immunoglobulin (Ig) G1 monoclonal antibody that blocks and downregulates EGFR and mediates immune antitumor mechanisms.[1-3] Cetuximab is particularly effective in combination with other therapeutic modalities. Cetuximab improves survival in squamous cell carcinoma of the head and neck when added to radiotherapy in locally advanced disease and when combined with chemotherapy in recurrent/metastatic disease.[4,5] Cetuximab is effective both as monotherapy and with chemotherapy in metastatic colorectal cancer (mCRC).[6-8] Results from the phase III trial First-Line Erbitux in Lung Cancer (FLEX) demonstrated that cetuximab improves overall survival (OS) significantly when added to a platinum doublet in patients with non–small-cell lung cancer (NSCLC) with EGFR-positive tumors by immunohistochemistry (IHC).[9]

BMS099, a phase III trial designed in support of FLEX, investigated cetuximab plus first-line taxane/carboplatin (Paraplatin; Bristol-Myers Squibb, New York, NY; T/C) in advanced NSCLC without restrictions by EGFR expression or histologic subtype. The primary end point—progression-free survival (PFS) assessed by an independent radiologic review committee (IRCC)—did not differ significantly between treatments; overall response rate (ORR) was significantly increased; and OS was greater with cetuximab but had a nonsignificant difference.[10] The activity observed, particularly the OS increase, was consistent in magnitude with the significant survival improvement achieved with cetuximab plus cisplatin (Platinol; Bristol-Myers Squibb, New York, NY) and vinorelbine (Navelbine; GlaxoSmithKline, Research Triangle Park, NC) in the FLEX trial.[9]

Patient selection on the basis of molecular markers predictive of clinical activity may enhance the risk-to-benefit ratio of cetuximab substantially. On the basis of exploratory subset studies of EGFR inhibitors in NSCLC, various candidate predictive markers have emerged.

Presence of *K-Ras* mutations seems to confer lack of benefit from tyrosine kinase inhibitor (TKI) –based therapy in NSCLC[11-13] and also from cetuximab in mCRC.[6,7,14] Compared with wild-type *EGFR*, activating *EGFR* mutations—present on tumors of approximately 10% of white and up to 50% of Asian patients with advanced NSCLC—confer higher sensitivity to TKIs.[11,15-17] Preliminary studies in mCRC and NSCLC suggest that this factor is not a determinant of cetuximab benefit.[18]

EGFR protein expression detected by IHC was the first molecular selection factor in EGFR inhibitor trials, but it has not been consistently associated with clinical benefit in TKI studies in NSCLC[17,19-24] or in cetuximab studies in mCRC.[25,26] This has mostly been attributed to technical shortcomings, such as the lack of standardization and sensitivity limitations of the IHC assay.[27]

Clinical benefit from TKI therapy has been repeatedly associated with *EGFR* gene copy number assessed by fluorescent in situ hybridization (FISH).[12,17,19,28] However, results are not consistent in all studies evaluating this marker.[29-32]

The present report details a retrospective, correlative analysis of candidate predictive markers in the BMS099 trial to identify factors that might help select patients and enhance the therapeutic index of cetuximab in advanced NSCLC.

METHODS

Study Design and Patients

BMS099 was a multicenter, open-label, phase III trial that enrolled patients with stage IIIB (pleural effusion) or stage IV NSCLC regardless of histology or EGFR expression. Patients received paclitaxel (Taxol; Bristol-Myers Squibb, New York, NY) or docetaxel (Taxotere; sanofi-aventis, Bridgewater, NJ) at investigator's discretion plus carboplatin every 3 weeks for up to six cycles. Cetuximab was given until progression or unacceptable toxicity occurred. PFS assessed by an IRCC was the primary end point; response rate and OS were secondary end points. BMS099 was conducted according to the Declaration of Helsinki and was approved by institutional review boards at all participating centers. All patients provided written informed consent (CONSORT diagram, Fig 1).[10]

Tissue Specimens

Formalin-fixed, paraffin-embedded tissue (FFPET) samples, as blocks or as 5-μm–thick unstained sections, were requested from the most recent diagnostic tumor biopsy available. No predetermined number of study participants was required, as participation in the correlative analysis was optional. All samples were evaluated for tumor content with hematoxylin and eosin to evaluate the presence of tumor.

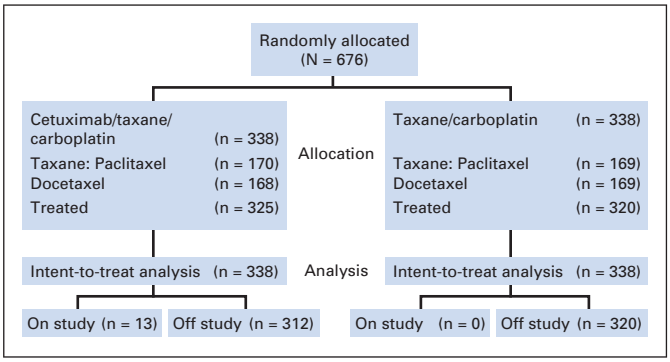


Fig 1. CONSORT diagram.

Molecular Analysis

Mutation analyses were performed on total genomic DNA extracted with the QIAamp DNA Mini Kit (Qiagen, Valencia, CA). *K-Ras* gene exon 2 and *EGFR* gene exons 18 to 21 were sequenced bidirectionally, as described before.[13-15,25] *EGFR* copy number measurement by FISH was performed as previously described[28,33]; samples were classified as *EGFR* FISH-positive (ie, high polysomy or gene amplification) or negative (ie, normal copy number) by using the Colorado scoring system.[27] EGFR protein expression was determined by IHC with the PharmDx Kit (Dako, Carpinteria, CA). Samples were scored as positive if one or more tumor cells showed staining. All correlative analyses were blinded to treatment assignment, patient characteristics, and outcomes.

Statistics

All randomized participants with an evaluable FFPET sample were included. A statistical analysis plan prespecified all analyses before experimental assessments were performed. Each biomarker was analyzed separately. *P* values were not adjusted for multiplicity.

For PFS and OS, two-sided, $\alpha = .05$, log-rank tests were used to assess differences among patients with different biomarker status for each treatment arm separately and treatment effect in patients with the same biomarker status. A Cox proportional hazards model was used to assess interaction between treatment effect and biomarker status, and both of the main effect terms were in the model. Estimates of the hazard ratio (HR) of the interaction, a two-sided, 95% CI of the HR, and *P* values were reported. ORR and 95% CIs were calculated for treatment arms by biomarker status with Fisher's exact method.

RESULTS

Tissue Availability

FFPET specimens were available from 225 (33.3%) of 676 randomly assigned patients. Evaluable tumor-containing samples were distributed as follows: 202 specimens (29.9%) for *K-Ras* mutation analysis, 166 (24.6%) for *EGFR* mutation analysis, 148 (21.9%) for EGFR protein expression by IHC, and 104 (15.4%) for FISH analysis of *EGFR* gene copy number. Baseline patient characteristics in each subset overall and between treatment groups in general were comparable to the entire BMS099 cohort (Table 1 and Appendix Tables A1 and A2, online only).

Patient Outcomes

In the intent-to-treat (ITT) analysis, cetuximab did not extend PFS (HR, 0.90; 95% CI, 0.76 to 1.07; stratified log-rank $P = .24$) or OS (HR, 0.89; 95% CI, 0.75 to 1.05; $P = .17$) significantly when added to T/C, but it improved ORR significantly (25.7% *v* 17.2%; $P = .007$).

Table 1. Demographic and Clinical Characteristics of Evaluable Patients

Characteristic	% of Patients by Data Set									
	All Patients (N = 676)		*K-Ras* (n = 202)		*EGFR* Mutation (n = 166)		EGFR IHC (n = 148)		*EGFR* FISH (n = 104)	
	Cet + T/C (n = 338)	T/C (n = 338)	Cet + T/C (n = 98)	T/C (n = 104)	Cet + T/C (n = 79)	T/C (n = 87)	Cet + T/C (n = 77)	T/C (n = 71)	Cet + T/C (n = 53)	T/C (n = 51)
Age, years										
Median	64	65	64	67	64	67	63	67	63	67
Range	37-87	34-85	45-84	34-83	45-84	34-83	45-83	34-83	46-78	34-83
≥ 65	49.4	51.2	46.9	58.6	46.8	59.8	42.9	62.0	45.3	58.8
Male sex	56.8	60.4	56.1	58.6	59.5	59.8	55.8	54.9	56.6	62.8
Ethnicity										
White	87.6	88.8	89.8	91.4	87.3	89.7	87.0	94.4	94.3	88.2
Black	7.4	7.1	8.2	5.8	10.1	6.9	10.4	4.2	5.7	7.8
Asian	1.8	3.0	0	2.9	0	3.5	0	1.4	0	3.9
Other	3.3	1.2	2.0	0	2.5	0	2.6	0	0	0
Histology										
Nonsquamous	63.0	65.1	70.4	65.4	73.4	67.8	74.0	59.2	71.7	62.8
Squamous	19.8	19.2	19.4	22.1	20.3	17.2	19.5	25.4	18.9	25.5
Unknown	17.2	15.7	10.2	12.5	6.3	14.9	6.5	15.5	9.4	11.8
ECOG PS										
0	32.5	33.7	37.8	36.5	35.4	34.5	40.3	35.2	43.4	27.5
1	65.4	65.1	61.2	62.5	63.3	64.4	58.4	63.4	56.6	70.6
2	1.2	0.6	1.0	1.0	1.3	1.2	1.3	1.4	0	2.0
Smoking										
Never	8.3	7.4	7.1	8.7	7.6	9.2	6.5	7.0	5.7	11.8
Former	63.3	67.8	68.4	69.2	65.8	70.1	67.5	71.8	64.2	64.4
Current	28.4	24.9	24.5	22.1	26.6	20.7	26.0	21.1	30.2	26.9

Abbreviations: EGFR, epidermal growth factor receptor; IHC, immunohistochemistry; FISH, fluorescent in situ hybridization; Cet, cetuximab; T/C, taxane/carboplatin; ECOG PS, Eastern Cooperative Oncology Group performance status.

Outcomes for some of the biomarker-evaluable cohorts were not fully representative of the total ITT population (Table 2; Data Supplement table, online only).

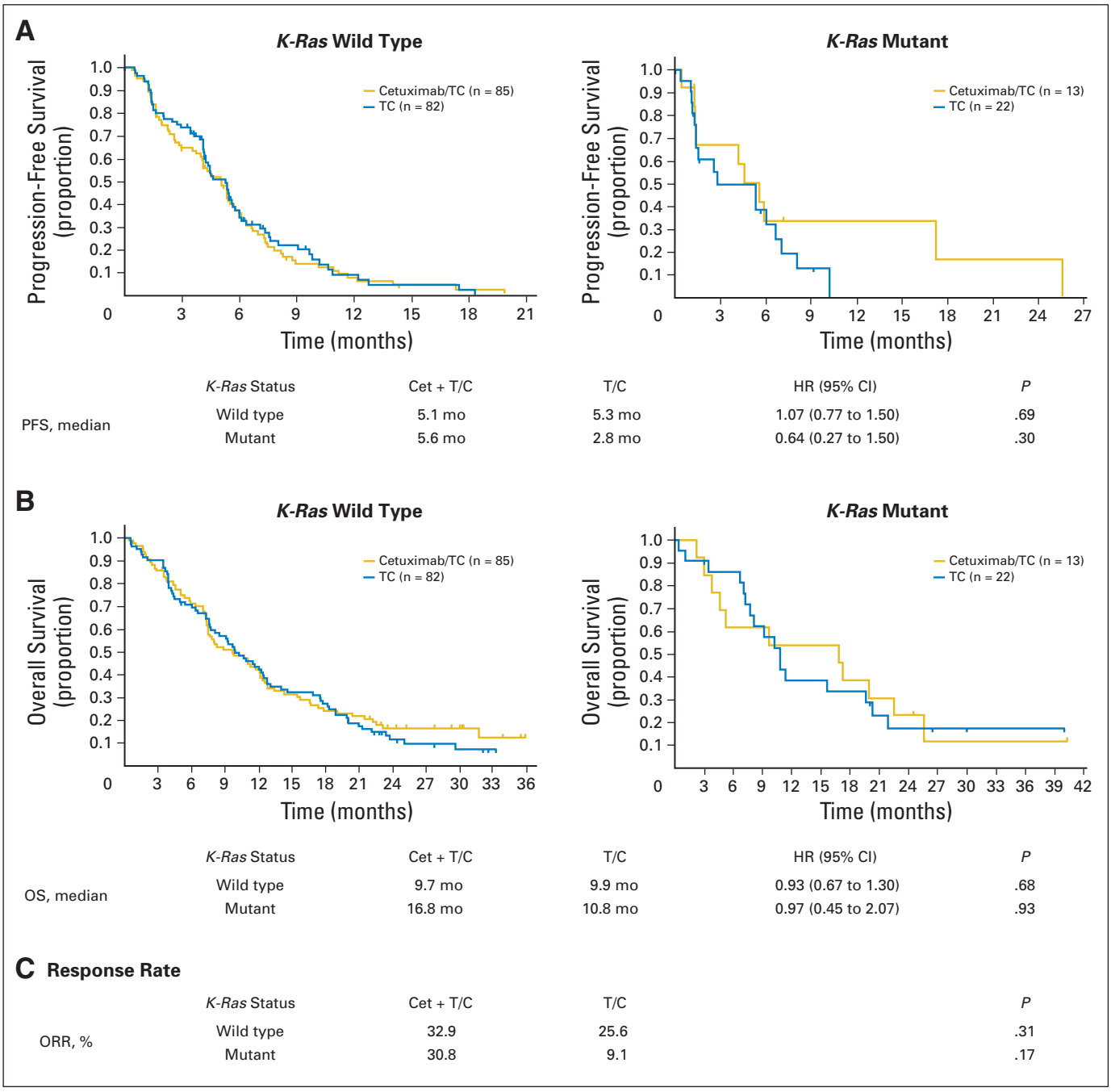
K-Ras *Mutation Status*

Thirty-five (17.3%) of 202 patients had *K-Ras* mutations; of these, 13 patients were randomly assigned to cetuximab plus T/C, and 22 patients were randomly assigned to T/C. Adding cetuximab to T/C did not significantly affect median PFS in patients with *K-Ras* wild-type tumors (5.1 *v* 5.3 months; HR, 1.07; 95% CI, 0.77 to 1.50; $P = .69$) or in the group with mutated *K-Ras* (5.6 *v* 2.8 months; HR, 0.64; 95% CI, 0.27 to 1.50; $P = .30$; Fig 2A). Combining cetuximab with T/C did not result in significant OS differences in the wild-type group (9.7 *v* 9.9 months; HR, 0.93; 95% CI, 0.67 to

Table 2. Outcomes in Evaluable Patients

Outcome	Data Set									
	All Patients (N = 676)		*K-Ras* (n = 202)		*EGFR* Mutation (n = 166)		EGFR IHC (n = 148)		*EGFR* FISH (n = 104)	
	Cet + T/C	T/C	Cet + T/C	T/C	Cet + T/C	T/C	Cet + T/C	T/C	Cet + T/C	T/C
PFS										
Median, months	4.0	4.2	5.1	5.3	5.4	5.4	4.6	5.3	4.5	5.3
HR	0.90		0.97		0.98		1.08		1.00	
95% CI	0.76 to 1.07		0.71 to 1.31		0.70 to 1.37		0.75 to 1.55		0.65 to 1.53	
P	.24		.83		.88		.68		1.00	
OS										
Median, months	9.7	8.4	9.8	10.2	10.9	10.6	8.3	9.8	8.3	10.8
HR	0.89		0.95		0.98		1.09		1.33	
95% CI	0.75 to 1.05		0.70 to 1.28		0.71 to 1.37		0.76 to 1.54		0.88 to 2.02	
P	.17		.71		.91		.65		.18	
ORR, %	25.7	17.2	32.7	22.1	34.2	20.7	29.9	22.5	34.0	19.3
95% CI	21.2 to 30.7	13.3 to 21.6	23.5 to 42.9	14.6 to 31.3	23.9 to 45.7	12.7 to 30.7	20.0 to 41.4	13.5 to 34.0	21.5 to 48.3	9.8 to 33.1

Abbreviations: EGFR, epidermal growth factor receptor; IHC, immunohistochemistry; FISH, fluorescence in situ hybridization; Cet, cetuximab; T/C, taxane/carboplatin; PFS, progression-free survival; HR, hazard ratio; OS, overall survival; ORR, overall response rate.



	K-Ras Status	Cet + T/C	T/C	HR (95% CI)	*P*
PFS, median	Wild type	5.1 mo	5.3 mo	1.07 (0.77 to 1.50)	.69
	Mutant	5.6 mo	2.8 mo	0.64 (0.27 to 1.50)	.30

	K-Ras Status	Cet + T/C	T/C	HR (95% CI)	*P*
OS, median	Wild type	9.7 mo	9.9 mo	0.93 (0.67 to 1.30)	.68
	Mutant	16.8 mo	10.8 mo	0.97 (0.45 to 2.07)	.93

C Response Rate

	K-Ras Status	Cet + T/C	T/C	*P*
ORR, %	Wild type	32.9	25.6	.31
	Mutant	30.8	9.1	.17

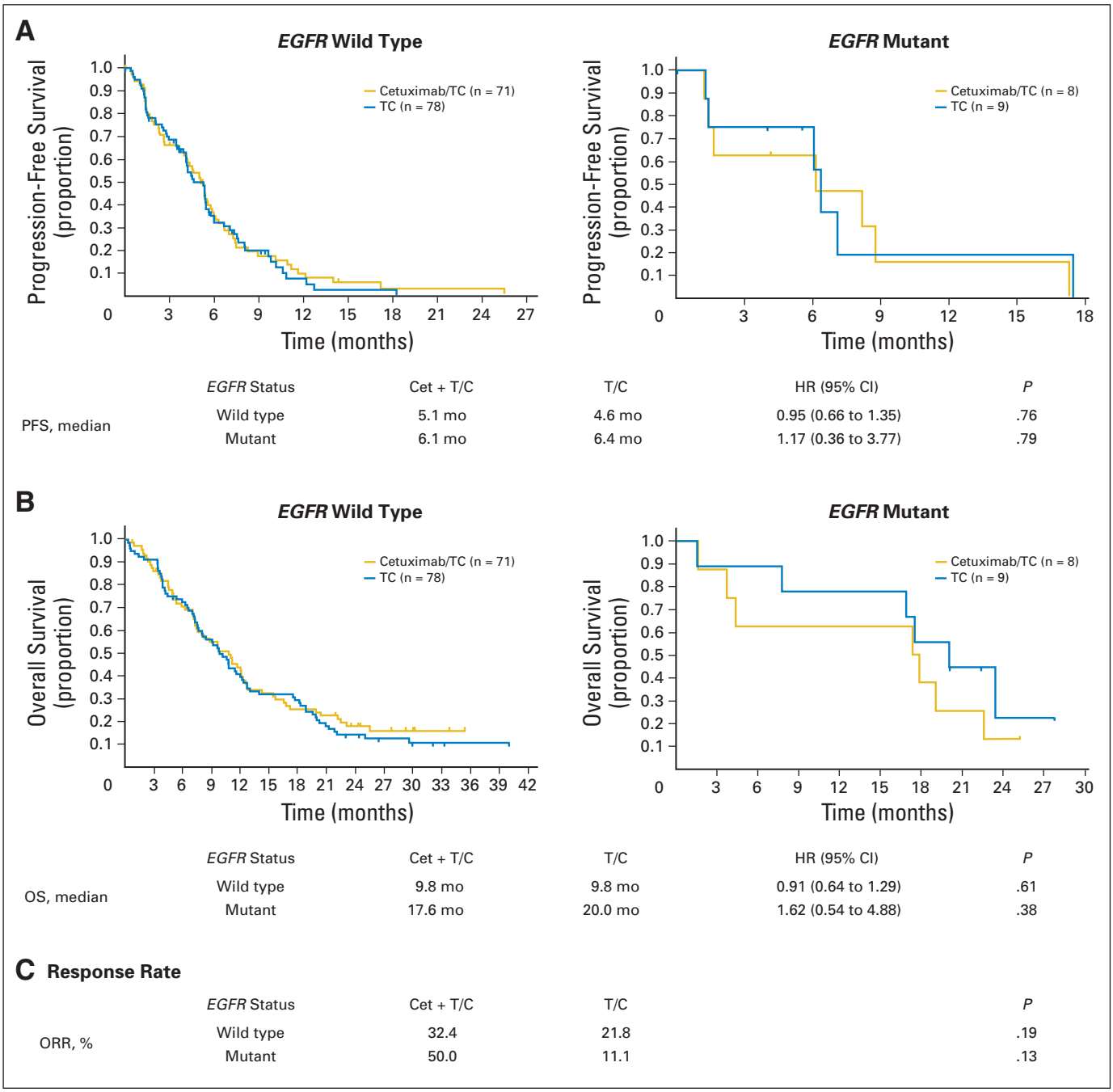
Fig 2. *K-Ras* mutation analysis. (A) Progression-free survival (PFS); (B) overall survival (OS); (C) response rate. Cet, cetuximab; T/C, taxane/carboplatin; HR, hazard ratio; ORR, overall response rate.

1.30; $P = .68$) or in the mutated *K-Ras* subset (16.8 *v* 10.8 months; HR, 0.97; 95% CI, 0.45 to 2.07; $P = .93$; Fig 2B).

In patients with mutated *K-Ras,* ORR was higher with cetuximab plus T/C than with T/C alone (30.8% *v* 9.1%; Fig 2C). ORR differences between treatments did not reach statistical significance for patients with either *K-Ras* mutation or wild type ($P = .17$ and 0.31, respectively). The association between *K-Ras* mutational status and ORR was not significant ($P = .19$).

EGFR *Mutational Status*

Seventeen (10.2%) of 166 evaluable patients had *EGFR* mutations; eight of these patients were from the cetuximab-plus-T/C arm, and nine patients were from the T/C arm. Adding cetuximab to T/C did not significantly affect PFS in patients with wild type (5.1 *v* 4.6 months; HR, 0.95; 95% CI, 0.66 to 1.35; $P = .76$) or in those with *EGFR* mutations (6.1 *v* 6.4 months; HR, 1.17; 95% CI, 0.36 to 3.77; $P = .79$; Fig 3A). Adding cetuximab to T/C did not affect OS in



	EGFR Status	Cet + T/C	T/C	HR (95% CI)	*P*
PFS, median	Wild type	5.1 mo	4.6 mo	0.95 (0.66 to 1.35)	.76
	Mutant	6.1 mo	6.4 mo	1.17 (0.36 to 3.77)	.79

	EGFR Status	Cet + T/C	T/C	HR (95% CI)	*P*
OS, median	Wild type	9.8 mo	9.8 mo	0.91 (0.64 to 1.29)	.61
	Mutant	17.6 mo	20.0 mo	1.62 (0.54 to 4.88)	.38

C Response Rate

	EGFR Status	Cet + T/C	T/C	*P*
ORR, %	Wild type	32.4	21.8	.19
	Mutant	50.0	11.1	.13

Fig 3. *EGFR* mutation analysis. (A) Progression-free survival (PFS); (B) overall survival (OS); (C) response rate. Cet, cetuximab; T/C, taxane/carboplatin; HR, hazard ratio; ORR, overall response rate.

patients with wild type (9.8 *v* 9.8 months; HR, 0.91; 95% CI, 0.64 to 1.29; $P = .61$) or patients with *EGFR* mutations (17.6 *v* 20.0 months; HR, 1.62; 95% CI, 0.54 to 4.88; $P = .38$; Fig 3B).

In the entire evaluable subset, OS tended to be longer in patients with mutated *EGFR* compared with those with wild-type *EGFR* (HR, 0.61; $P = .09$). This trend was more apparent in the T/C group (HR, 0.46; $P = .06$) than in the cetuximab-plus-T/C group (HR, 0.84; $P = .66$).

In the small subgroup with *EGFR* mutations, the ORR tended to be higher in patients receiving cetuximab plus T/C (50% *v* 11.1%; $P = .13$); a similar pattern was found in the *EGFR* wild-type group (32.4% *v* 21.8%; Fig 3C). The association between *EGFR* mutational status and ORR was not significant ($P = .82$).

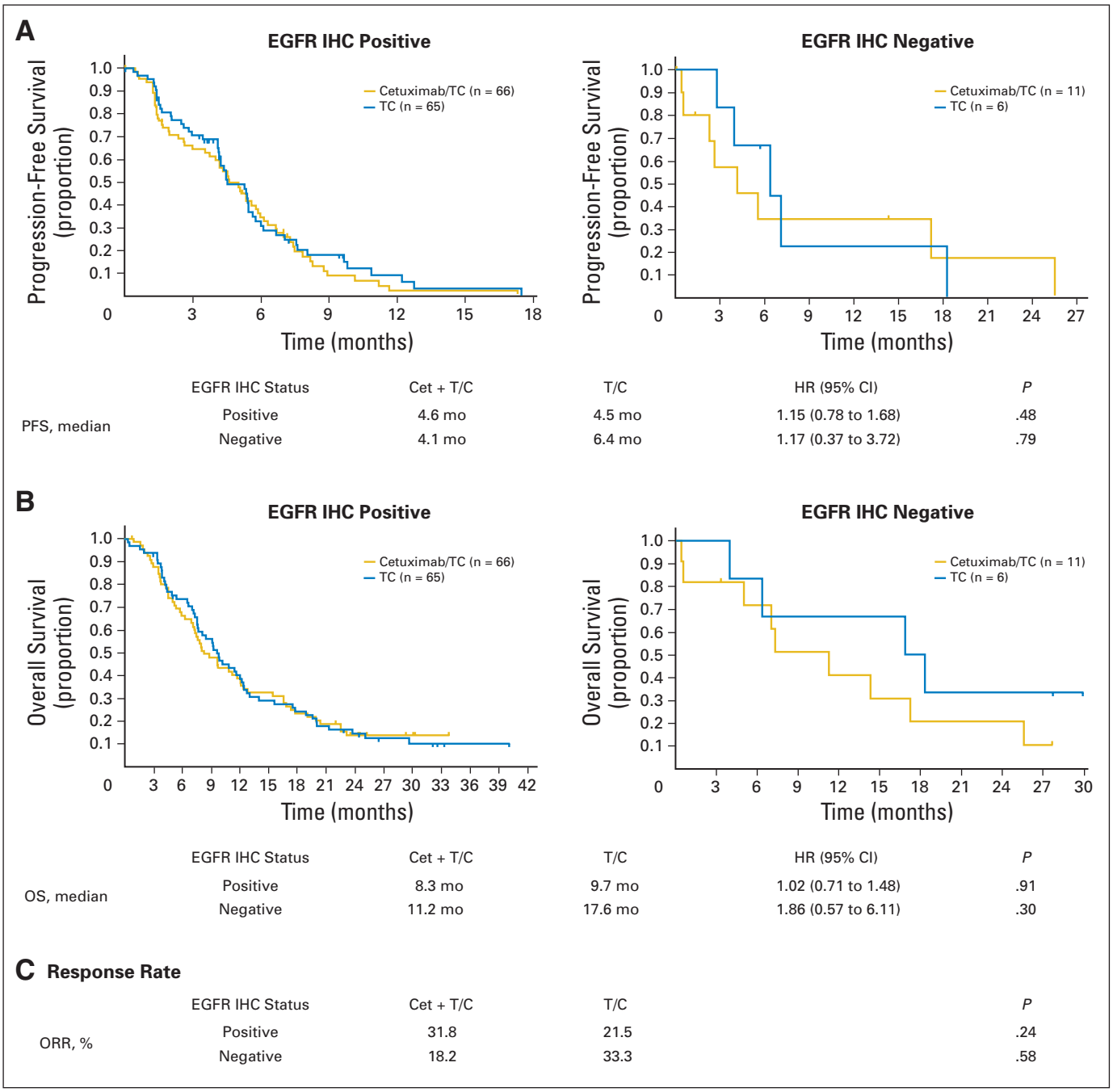
EGFR Protein Expression

Seventeen (11.5%) of 148 patients had tumors that did not express EGFR when assessed by IHC; 11 of these patients were randomly assigned to cetuximab plus T/C, and six patients were on T/C alone. PFS results were comparable in the EGFR-positive subgroup (median, 4.6 *v* 4.5 months; HR, 1.15; 95% CI, 0.78 to 1.68; $P = .48$) and in the

subset with EGFR-negative tumors (median, 4.1 *v* 6.4 months; HR, 1.17; 95% CI, 0.37 to 3.72; $P = .79$; Fig 4A). Regardless of treatment, patients with EGFR-positive tumors had significantly shorter PFS than those with EGFR-negative tumors (4.6 *v* 5.5 months; HR, 1.96; $P = .048$). This profile was evident with both T/C (HR, 2.03; $P = .17$) and cetuximab plus T/C (HR, 1.81; $P = .15$).

Adding cetuximab to T/C did not significantly affect OS in patients with EGFR-positive tumors (median, 8.3 *v* 9.7 months; HR, 1.02; 95% CI, 0.71 to 1.48; $P = .91$) or in the small subset of patients with EGFR-negative tumors (median, 11.2 *v* 17.6 months; HR, 1.86; 95% CI, 0.57 to 6.11; $P = .30$; Fig 4B). OS did not differ significantly by EGFR IHC status in the entire cohort (HR, 1.27; $P = .41$). In the T/C group, OS seemed shorter for the patients who had EGFR-positive tumors compared with those who had EGFR-negative tumors, but the difference was not statistically significant (HR, 1.86; $P = .22$). No difference by EGFR IHC status was seen in the cetuximab plus T/C group (HR, 1.00; $P = .99$).

No significant ORR differences between treatment arms were seen either in patients with EGFR-positive tumors (31.8% *v* 21.5%; $P = .24$) or with EGFR-negative tumors (18.2% *v* 33.3%; $P = .58$)



	EGFR IHC Status	Cet + T/C	T/C	HR (95% CI)	*P*
PFS, median	Positive	4.6 mo	4.5 mo	1.15 (0.78 to 1.68)	.48
	Negative	4.1 mo	6.4 mo	1.17 (0.37 to 3.72)	.79
OS, median	Positive	8.3 mo	9.7 mo	1.02 (0.71 to 1.48)	.91
	Negative	11.2 mo	17.6 mo	1.86 (0.57 to 6.11)	.30

	EGFR IHC Status	Cet + T/C	T/C	*P*
ORR, %	Positive	31.8	21.5	.24
	Negative	18.2	33.3	.58

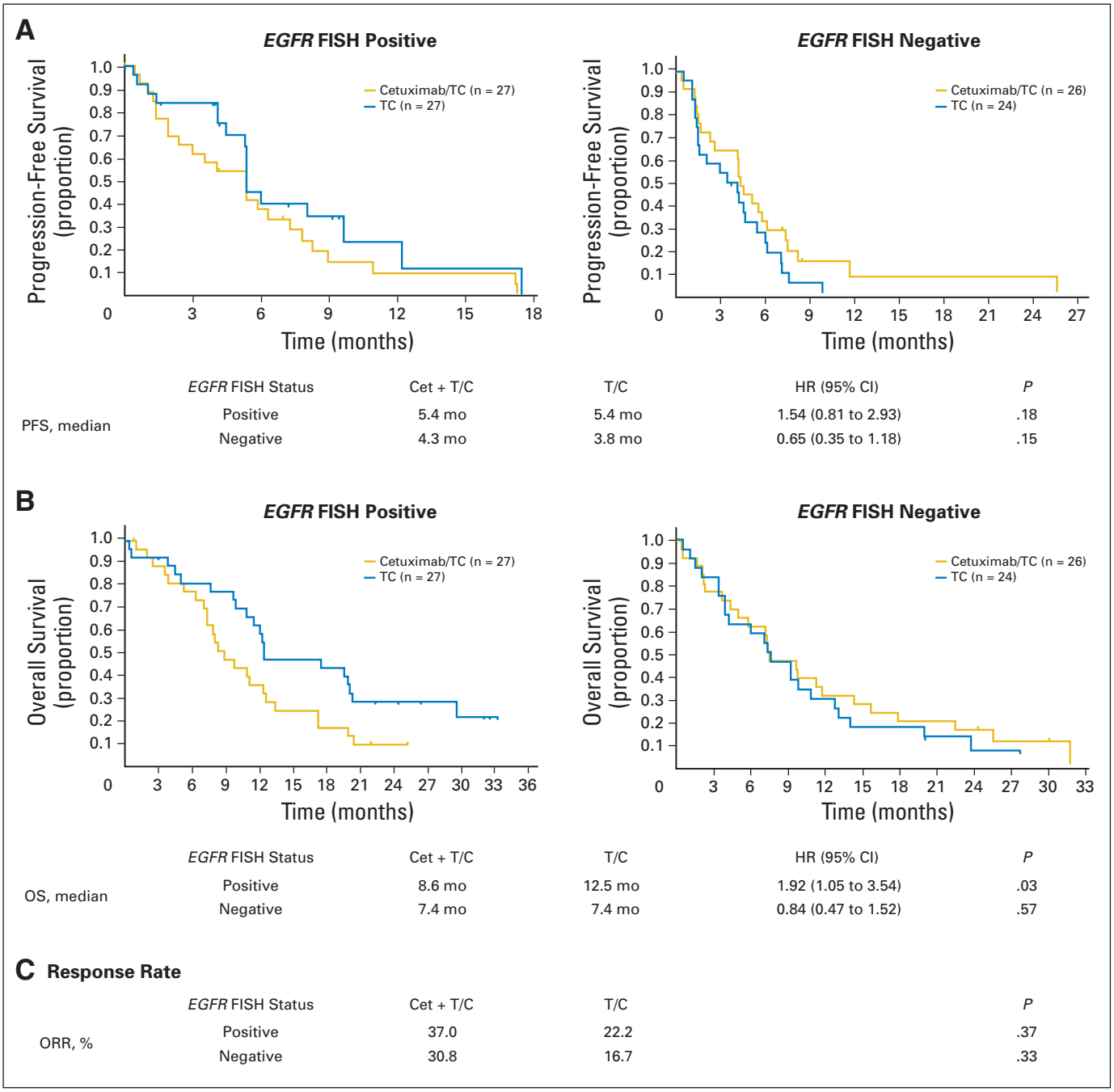
Fig 4. Analysis of *EGFR* expression by immunohistochemistry (IHC). (A) Progression-free survival (PFS); (B) overall survival (OS); (C) response rate. Cet, cetuximab; T/C, taxane/carboplatin; HR, hazard ratio; ORR, overall response rate.

or in the comparisons according to EGFR status in patients treated with cetuximab plus T/C ($P = .49$) or T/C alone ($P = .61$; Fig 4C). The association between EGFR expression and ORR was not significant ($P = .71$).

EGFR *Gene Copy Number by FISH*

Fifty-four of 104 evaluable patients (51.9%) were *EGFR* FISH positive, including 47 with high polysomy and seven with gene amplification. The FISH-positive subset included 27 patients assigned to cetuximab plus T/C and 27 patients assigned to T/C alone. The addition of cetuximab to T/C did not significantly affect PFS in the FISH-positive subset (median, 5.4 months in both arms; HR, 1.54; 95% CI, 0.81 to 2.93; $P = .18$) or in the FISH-negative subset (4.3 *v* 3.8 months; HR, 0.65; 95% CI, 0.35 to 1.18; $P = .15$; Fig 5A). In the cetuximab-plus-T/C group, no PFS difference was evident between patients in the FISH-positive and FISH-negative subsets (HR, 0.99; $P = .97$). Patients with FISH-positive tumors treated with T/C had significantly longer PFS than those with FISH-negative tumors (HR, 1.41; $P = .007$).

Patients with *EGFR* FISH-positive tumors had significantly shorter OS with cetuximab plus T/C than with T/C alone (median,



	EGFR FISH Status	Cet + T/C	T/C	HR (95% CI)	*P*
PFS, median	Positive	5.4 mo	5.4 mo	1.54 (0.81 to 2.93)	.18
	Negative	4.3 mo	3.8 mo	0.65 (0.35 to 1.18)	.15
OS, median	Positive	8.6 mo	12.5 mo	1.92 (1.05 to 3.54)	.03
	Negative	7.4 mo	7.4 mo	0.84 (0.47 to 1.52)	.57

	EGFR FISH Status	Cet + T/C	T/C	*P*
ORR, %	Positive	37.0	22.2	.37
	Negative	30.8	16.7	.33

Fig 5. Analysis of *EGFR* gene copy number by fluorescent in situ hybridization (FISH). (A) Progression-free survival (PFS); (B) overall survival (OS); (C) response rate. T/C, taxane/carboplatin; HR, hazard ratio; ORR, overall response rate.

8.6 *v* 12.5 months; HR, 1.92; 95% CI, 1.05 to 3.54; $P = .03$; Fig 5B), whereas OS did not differ by treatment in patients with FISH-negative tumors (median, 7.4 months in both groups; HR, 0.84; 95% CI, 0.47 to 1.52; $P = .57$). Patients with FISH-positive tumors had longer OS than those with FISH-negative tumors when treated with chemotherapy alone (HR, 0.48; $P = .017$) but not when treated with cetuximab plus T/C (HR, 1.07; $P = .81$).

In the *EGFR* FISH-evaluable population overall, the ORR was numerically higher with cetuximab plus T/C than with T/C alone (34.0% *v* 24.0%); similar profiles were seen in patients with FISH-positive (37.0% *v* 22.2%; $P = .37$) and FISH-negative (30.8% *v* 16.7%; $P = .33$) tumors (Fig 5C).

DISCUSSION

Cetuximab prolonged survival in patients with NSCLC when combined with platinum-based chemotherapy in the phase III FLEX trial.[9] BMS099, the supportive study to FLEX, did not reach its primary PFS end point, though ORR improvements and a favorable OS trend were observed. These results highlight the importance of predictive biomarkers to identify patients likely to benefit from cetuximab and to optimize its use. BMS099 provides the first correlative analyses of a randomized, controlled trial of cetuximab in advanced NSCLC and provides valuable preliminary evidence on potential predictive markers for patient selection. In this study, benefit from cetuximab did not appear to be associated with *K-Ras* or *EGFR* mutations, EGFR protein expression, or *EGFR* gene copy number.

Even without mandatory tissue collection in the original BMS099 protocol, the tissue collection rate was 30%. However, the limited total number of samples evaluable for each individual test warrants cautious interpretation of all statistical analyses. Nonetheless, despite low sample numbers that preclude firm conclusions, the consistency between BMS099 and other recent correlative studies is noteworthy, which indicates that cetuximab benefit is not likely to be associated with any of the markers initially considered obvious candidates.

This study showed no significant treatment-specific interactions between the presence of *K-Ras* mutations (in 17.3% of patients) and the outcomes evaluated. Differences that favored the addition of cetuximab compared with T/C alone in the *K-Ras* mutant subgroup in terms of PFS (HR, 0.64; $P = .30$), OS (HR, 0.97; $P = .93$), and ORR (30.8% *v* 9.1%; $P = .17$) were consistent with those observed in patients with wild-type *K-Ras* and in the overall study population.

The correlative analysis of 395 samples from FLEX (frequency of *K-Ras* mutation, 19%) reported similar findings, which shows that the OS benefit of cetuximab was not affected by *K-Ras* mutation status.[34] In addition, correlative analyses of SWOG 0342 (investigating cetuximab/carboplatin/paclitaxel) and SWOG 0536 (investigating cetuximab/bevacizumab [Avastin; Genentech, San Francisco, CA]/carboplatin/paclitaxel), also showed comparable clinical activity of either combination regardless of *K-Ras* mutation status.[35] These findings contrast sharply with those in mCRC,[6,7,14] and the biologic reasons for this divergence are not completely understood. *K-Ras* mutations are less prevalent in NSCLC (17.3%), in which tumors are more histologically diverse, than in mCRC (33% to 40%).[11,12,36,37] Smoking history and histologic subtype seem to affect the type of *K-Ras* mutation found in NSCLC.[38] Overall dependence on EGFR signaling for growth and survival, and subsequent EGFR inhibition effect, may not be the same in NSCLC and CRC. In addition, cetuximab could be acting via antibody-dependent, cell-mediated cytotoxicity in NSCLC tumors, as well as by EGFR blockade. Even without a precise explanation, it is clear that *K-Ras* mutations have different effects in advanced NSCLC and mCRC. Consequently, tumor-specific approaches are needed for clinical treatment decisions in these malignancies.

In the IHC analysis of EGFR expression, only 17 patients (11.5%) were classified as EGFR negative, which is consistent with previous reports.[9,17,19-24] Even with the caveat of low numbers, no outcome discrepancies were seen in the EGFR-positive and EGFR-negative subsets, for which HRs for PFS and OS were mostly comparable. SWOG 0536 and SWOG 0342 results also are consistent with this observation, which points to the lack of effect of EGFR expression on cetuximab activity.[39] Additionally, although it is not possible to search for companion evidence from the FLEX trial, for which EGFR IHC-negative status was an exclusion criterion,[9] the consistency in outcomes between BMS099 and FLEX argues that EGFR IHC status is irrelevant to cetuximab benefit in NSCLC, similar to the findings in mCRC.[25,26,40]

This analysis does not support *EGFR* FISH as a marker with predictive value for cetuximab activity. Even with a relatively small sample, *EGFR* FISH-positive status was found in 52% of tumors, a rate comparable to other reports in advanced NSCLC.[12,19,29,30] Surprisingly, patients with FISH-positive disease appeared to have superior outcomes with T/C alone (OS HR, 1.92; $P = .03$). This finding may be erroneous, because the *EGFR* FISH-evaluable subset was not fully representative of the total ITT population. However, it is interesting to note that a study of the EGFR TKI gefitinib compared with single-agent chemotherapy showed a similar trend for superior outcomes with chemotherapy alone in patients with *EGFR* FISH-positive tumors.[29] Nonetheless, no significant, treatment-specific interactions were found in BMS099 between *EGFR* FISH status and either PFS or ORR. Again, similar results were found in the FLEX trial, in which 37% of the 279 samples analyzed were *EGFR* FISH positive, and FISH status did not affect the OS gains associated with cetuximab.[34] Interestingly, these findings contrast with data from SWOG 0342 that report longer PFS and OS with cetuximab in patients with FISH-positive versus FISH-negative tumors.[41] However, BMS099 and FLEX are randomized, controlled studies; SWOG 0342 provides an uncontrolled data set, in which a potentially prognostic effect may confound a predictive analysis. Results with TKIs for this biomarker are also conflicting: FISH-positive status correlates with outcomes in single-agent versus placebo-controlled trials,[17,19] but there is no correlation, or results seem mixed, in combination trials (e.g., the correlation with time to progression but not with response in TRIBUTE [Tarceva Responses in Conjunction with Paclitaxel and Carboplatin]).[30]

Cautious interpretation of *EGFR* FISH data is warranted, as the relevance of *EGFR* FISH positivity is still unclear. With the available published evidence, *EGFR* gene copy number by FISH could be interpreted as a prognostic marker or as a potentially predictive marker of the efficacy of chemotherapy rather than of cetuximab.[42] Prospective studies may help to additionally clarify its role.

Finally, significant correlations were not observed between *EGFR* mutation status and specific outcomes with cetuximab. Accordingly, unlike EGFR TKIs,[43,44] *EGFR* mutation status is not a predictive biomarker for cetuximab activity in NSCLC. This finding aligns with

the presumed effect of *EGFR* tyrosine-kinase domain mutations, which enhance the intracellular binding of TKIs but are not expected to affect extracellular blockade by an antibody.[16,17]

Continuing studies are needed to identify markers predictive of cetuximab benefit in NSCLC. Expression levels of EGFR ligand genes, particularly amphiregulin and epiregulin, are strongly associated with cetuximab benefit in mCRC.[45,46] Absence of PTEN (a downstream negative regulator of EGFR signaling) may be associated with cetuximab resistance in mCRC.[47,48] Evidence from SWOG 0342 and 0536, as well as from the BR.21 study of erlotinib, suggests that markers related to the epithelial-mesenchymal transition, such as vimentin or E-cadherin, may be clinically relevant.[39,49] Interest in all these markers is growing, even though information about them in NSCLC remains limited.

In conclusion, the results of this correlative study of cetuximab plus chemotherapy in NSCLC do not show that any of the biomarkers analyzed have a statistically significant effect on cetuximab benefit. Even *K-Ras* mutations, strongly associated with lack of cetuximab benefit in mCRC, fail to show a similar correlation in NSCLC. These data must be interpreted with caution, considering the retrospective nature of the analysis and the limited sample size. Additional studies are needed to identify predictive biomarkers of cetuximab benefit in NSCLC, including alternative candidate markers and de novo exploratory approaches, such as gene expression profiling on microarrays.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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