original article

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Evaluation of *EGFR* gene copy number as a predictive biomarker for the efficacy of cetuximab in combination with chemotherapy in the first-line treatment of recurrent and/or metastatic squamous cell carcinoma of the head and neck: EXTREME study

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Background: The phase III EXTREME study demonstrated that combining cetuximab with platinum/5-fluorouracil (5-FU) significantly improved overall survival in the first-line treatment of patients with recurrent and/or metastatic squamous cell carcinoma of the head and neck (R/M SCCHN) compared with platinum/5-FU alone. The aim of this investigation was to evaluate elevated tumor *EGFR* gene copy number as a predictive biomarker in EXTREME study patients.

Patients and methods: Dual-color FISH was used to determine absolute and relative *EGFR* copy number. Models of differing stringencies were used to score and investigate whether increased copy number was predictive for the activity of cetuximab plus platinum/5-FU.

Results: Tumors from 312 of 442 patients (71%) were evaluable by FISH and met the criteria for statistical analysis. A moderate increase in *EGFR* copy number was common, with high-level amplification of the gene occurring in a small fraction of tumors (~11%). Considering each of the models tested, no association of *EGFR* copy number with overall survival, progression-free survival or best overall response was found for patients treated with cetuximab plus platinum/5-FU.

Conclusion: Tumor *EGFR* copy number is not a predictive biomarker for the efficacy of cetuximab plus platinum/5-FU as first-line therapy for patients with R/M SCCHN.

Key words: cetuximab, copy number, EFGR, EXTREME, FISH, platinum/5-fluorouracil

introduction

The randomized phase III EXTREME study demonstrated that the addition of cetuximab to platinum/5-fluorouracil (5-FU) chemotherapy statistically significantly improved overall survival when given as first-line treatment to patients with recurrent and/or metastatic squamous cell carcinoma of the head and neck (R/M SCCHN) compared with platinum/5-FU alone (median 10.1 versus 7.4 months, hazard ratio 0.80, $P = 0.04$) [1]. The addition of cetuximab to platinum/5-FU also led to significant improvements in progression-free survival (PFS) and best overall response rate, which was approximately doubled. Safety analysis demonstrated that the combination was feasible, with a manageable side-effect profile. The 2.7-month median survival time benefit associated with the addition of this epidermal growth factor receptor (EGFR)-targeted monoclonal antibody to standard platinum-based chemotherapy represents the most significant advance in the treatment of the disease in this setting for ~30 years. These data complement an earlier study in locally advanced SCCHN which showed that the addition of cetuximab to radiotherapy conferred a long-term survival benefit compared with

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radiotherapy alone, the magnitude of which (9% absolute survival benefit at 5 years) was similar to that achievable in this setting with chemoradiotherapy [2–5].

Recent studies have shown that the clinical impact of EGFR-targeted therapies can be increased if treatment administration can be tailored to particular subpopulations of patients whose tumors have specific molecular alterations [6, 7]. Elevated gene copy number, which may arise within a tumor cell as the result of an increase in the numbers of chromosomes encoding the gene (polysomy) or may occur as a consequence of local amplification of a chromosomal region (gene amplification), is a somatic event with potential predictive utility. Increased copy number may indicate that a tumor is highly dependent on the activity of an amplified gene for continued proliferation and/or survival, a situation described as oncogene addiction [8]. In this case, the tumor may be particularly sensitive to anticancer agents that target the product of that gene and elevated copy number may consequently be a predictive biomarker, as exemplified by *ERBB2* gene amplification in breast cancer and sensitivity to trastuzumab [9]. Copy number can be evaluated in tumors and the two different causal genetic mechanisms can be distinguished through the use of dual-color FISH analysis incorporating a gene-specific probe combined with a centromere-specific probe for the chromosome encoding that gene.

The data on the impact of *EGFR* gene copy number status on cetuximab efficacy in metastatic colorectal cancer (mCRC) and non-small-cell lung cancer (NSCLC) is contradictory. While some studies reported an association of high *EGFR* gene copy number and improved outcome in mCRC and NSCLC patients receiving cetuximab [10–14], other studies failed to identify similar associations [15–17]. No data on *EGFR* gene copy number and cetuximab efficacy have so far been reported for SCCHN.

Expressed in 90%–100% of tumors, up-regulation of *EGFR* appears to be an early marker of SCCHN carcinogenesis [18–20], and high-level tumor expression has been correlated with poor clinical outcome [21]. Elevation of *EGFR* copy number is a characteristic somatic event that occurs in the development of this disease and may additionally be an indicator of poor prognosis [22, 23]. The aim of the current study was to investigate in the large relatively homogeneous population recruited for the randomized phase III EXTREME study whether elevated tumor *EGFR* copy number was predictive for the activity of cetuximab plus platinum/5-FU, administered as first-line therapy to patients with R/M SCCHN.

patients and methods

EXTREME study design

As previously reported [1], inclusion criteria included age ≥18 years, untreated R/M SCCHN, ineligibility for local therapy, Karnofsky Performance Score of ≥70% and adequate organ function. Patients were excluded if they had received prior surgery or radiotherapy within 4 weeks of study entry or prior systemic chemotherapy (apart from for locally advanced disease).

Patients were randomly assigned to receive every 3 weeks for up to six cycles either cisplatin, 100 mg/m^2 day 1, or carboplatin area under the curve of 5 day 1 (physician's choice); plus 5-FU infused at 1000 mg/m^2/day for 4 days either with or without cetuximab, administered at an initial dose of 400 mg/m^2 and then 250 mg/m^2 weekly, both during chemotherapy and subsequently as maintenance therapy until the occurrence of disease progression or unacceptable toxicity. The primary end point was overall survival. Secondary end points included PFS, best overall response, disease control, time-to-treatment failure, duration of response and safety.

collection and storage of patient material

All patients provided written informed consent for EGFR testing on tumor samples. All available formalin-fixed paraffin-embedded (FFPE) tumor tissue specimens from patients in the clinical study (blocks and slides) were analyzed at a central laboratory (Wuppertal Institute of Pathology, Wuppertal, Germany) according to a standard protocol.

FISH analysis

FISH analysis was carried out on deparaffinized 3- to 5-μm FFPE sections using the prepared solutions and protocol provided in the Histology FISH Accessory Kit (Dako, Denmark; see supplemental Methods, available at *Annals of Oncology* online). *EGFR* copy number was assessed using an *EGFR*/CEN-7 FISH probe mix (Dako). Fluorescence was visualized using a DM50000 B (Leica, Germany) fluorescence microscope with a DAPI filter and a Texas Red double filter. *EGFR* (normal location 7p11.2) signals appeared red and CEN-7 signals (probe homologous to the centromeric region of chromosome 7) appeared green.

statistical methods

The FISH investigation was a retrospectively planned exploratory analysis. For a patient to be included, a target of 100 evaluable (where a signal was present for both *EGFR* and CEN-7) cells and a minimum of 50 cells were to be assessed. Patients from the intention-to-treat (ITT) population with FISH assessments for at least 50 cells formed the FISH ITT population. For each analyzed cell, observed *EGFR*/CEN-7 signals were used to determine absolute and relative *EGFR* copy numbers. Average (mean) signal counts or ratios per patient were calculated.

Given the possibility that the most appropriate scoring system to assess the association of copy number changes and clinical outcome may vary according to the disease or the particular stage of disease, a series of different systems were used to define FISH-positive (elevated *EGFR* copy number) and FISH-negative (nonelevated *EGFR* copy number) status in R/M SCCHN, including five predefined *EGFR* enrichment models and the Colorado scoring system, previously developed for the analysis of *EGFR* copy number in NSCLC (Table 1) [12, 24].

EGFR enrichment models

EGFR enrichment models were evaluated in both treatment groups of the study. Five different models were developed using different thresholds to define each analyzed cell as FISH positive or negative. To derive a factor representative of the degree of heterogeneity across the tumor cells sampled for each patient, the percentage of FISH-positive tumor cells (of those analyzed) was then calculated for each patient and model (FISH score; ranging from 0% to 100%). For each model, for patients in each study arm, these values were used to construct scatter plots of survival time and PFS time versus the FISH score. These plots were subsequently assessed both visually and statistically (see supplemental Methods, available at *Annals of Oncology* online) in an attempt to identify a particular threshold value that allowed for a significant enrichment of patients with a survival benefit according to *EGFR* copy number status. For each model, for each arm, box plots of best overall response according to FISH score were also constructed and assessed visually to determine whether a clear correlation was apparent between tumor response and *EGFR* copy number.

Table 1. FISH scoring systems

Scoring systems and models	Definitions
EGFR enrichment model for evaluation of FISH status	
Model A	
FISH positive	*EGFR*/CEN-7 ratio ≥2 or presence of *EGFR* signal cluster
Model B	
FISH positive	*EGFR* signal count ≥3 or presence of *EGFR* signal cluster
Model C	
FISH positive	*EGFR* signal count ≥6 or presence of *EGFR* signal cluster
Model D	
FISH positive	*EGFR*/CEN-7 ratio ≥2 or presence of *EGFR* signal cluster or *EGFR* signal count ≥3
Model E	
FISH positive	*EGFR*/CEN-7 ratio ≥2 or presence of *EGFR* signal cluster or *EGFR* signal count ≥6
Colorado scoring system	
FISH positive	≥40% of cells display ≥4 *EGFR* counts or the presence of gene amplification, as defined by either Mean *EGFR*/CEN-7 ratio ≥2 >10% of cells displaying >15 *EGFR* counts >10% of the cells displaying the presence of loose or tight *EGFR* signal clusters or atypically large *EGFR* signals (*EGFR* cluster scored)

CEN-7, probe for centromeric region of human chromosome 7.

Colorado scoring system

EGFR copy number was also defined for patients in each study arm according to the previously established Colorado scoring system (Table 1). This system differed from the enrichment models in that it allowed the classification of tumors (rather than individual tumor cells) as either FISH positive or negative. The association of FISH status according to the Colorado system with clinical outcome was investigated using log-rank (PFS and overall survival) and Cochran–Mantel–Haenszel (response) tests.

results

patient population and material

Tumor tissue samples were available from 381 of the 442 (86%) patients in the ITT population of the EXTREME study. Samples from 312 patients (71%) were evaluable by FISH and met the criteria for statistical analysis (FISH ITT population; Table 2). Treatment arms were essentially balanced with respect to the number of evaluable samples, with 158 deriving from patients receiving cetuximab plus chemotherapy (71%) and 154 from those receiving chemotherapy alone (70%). The effects of treatment in relation to overall survival, PFS and best overall response were comparable for the ITT and FISH ITT populations (see supplemental Table 1, available at *Annals of Oncology* online).

Table 2. ITT patients assessed for *EGFR* tumor gene copy number by FISH

Patients, *n* (%)	Cetuximab + chemotherapy	Chemotherapy alone
Randomly assigned to treatment (ITT population)	222 (100)	220 (100)
FISH assessments not performed	28 (13)	33 (15)
FISH assessments performed	194 (87)	187 (85)
FISH results not available[a]	35 (16)	33 (15)
Assessment not possible for technical reasons	29 (13)	23 (10)
Excluded from statistical analysis (sample taken after first dose of cetuximab)	11 (5)	12 (5)
FISH results available	159 (72)	154 (70)
FISH results available for ≥50 cells (FISH ITT population)	158 (71)	154 (70)
FISH results for <50 cells	1 (0.5)	0

[a]Both reasons may apply.
ITT, intention to treat.

FISH analysis

Dual-color FISH analysis was carried out to evaluate for each tumor the number of signals in each cell related to *EGFR* and to the centromeric region of chromosome 7. Representative images from these assays are shown in Figure 1. The average numbers of *EGFR* and CEN-7 signals and the average ratio of *EGFR*/CEN-7 signals were calculated for the tumors of patients in each arm and in the overall FISH ITT population (Table 3). The decimal fraction of cells with *EGFR* signal clusters was also determined.

The distributions of the average signal counts for *EGFR* and CEN-7 and the *EGFR*/CEN-7 ratio were comparable between the two treatment groups. Tumor *EGFR* gene copy number was elevated in a substantial fraction of patients, with 40% of the FISH ITT population having average *EGFR* signal counts per cell of >3 and 11% of >5 (Table 3). The observed elevation of tumor *EGFR* gene copy number was due to both polysomy events (27% of patients had average CEN-7 signal counts of >3) and local amplification (12% of patients had an average cellular *EGFR*/CEN-7 ratio of >2). In 13% of patients, a fraction of tumor cells was scored as having strong localized *EGFR* amplification, such that individual signals could not be distinguished (clusters): 11% of patients had such clusters in ≥25% of tumor cells (Table 3).

As there was no known *EGFR* copy number threshold value that might be of predictive utility in this setting, a series of models with different stringencies were designed to provide definitions, which could be used to assign FISH status (Table 1). These models were then used to assess whether elevated *EGFR* copy number, as defined in each model, was predictive for cetuximab efficacy.

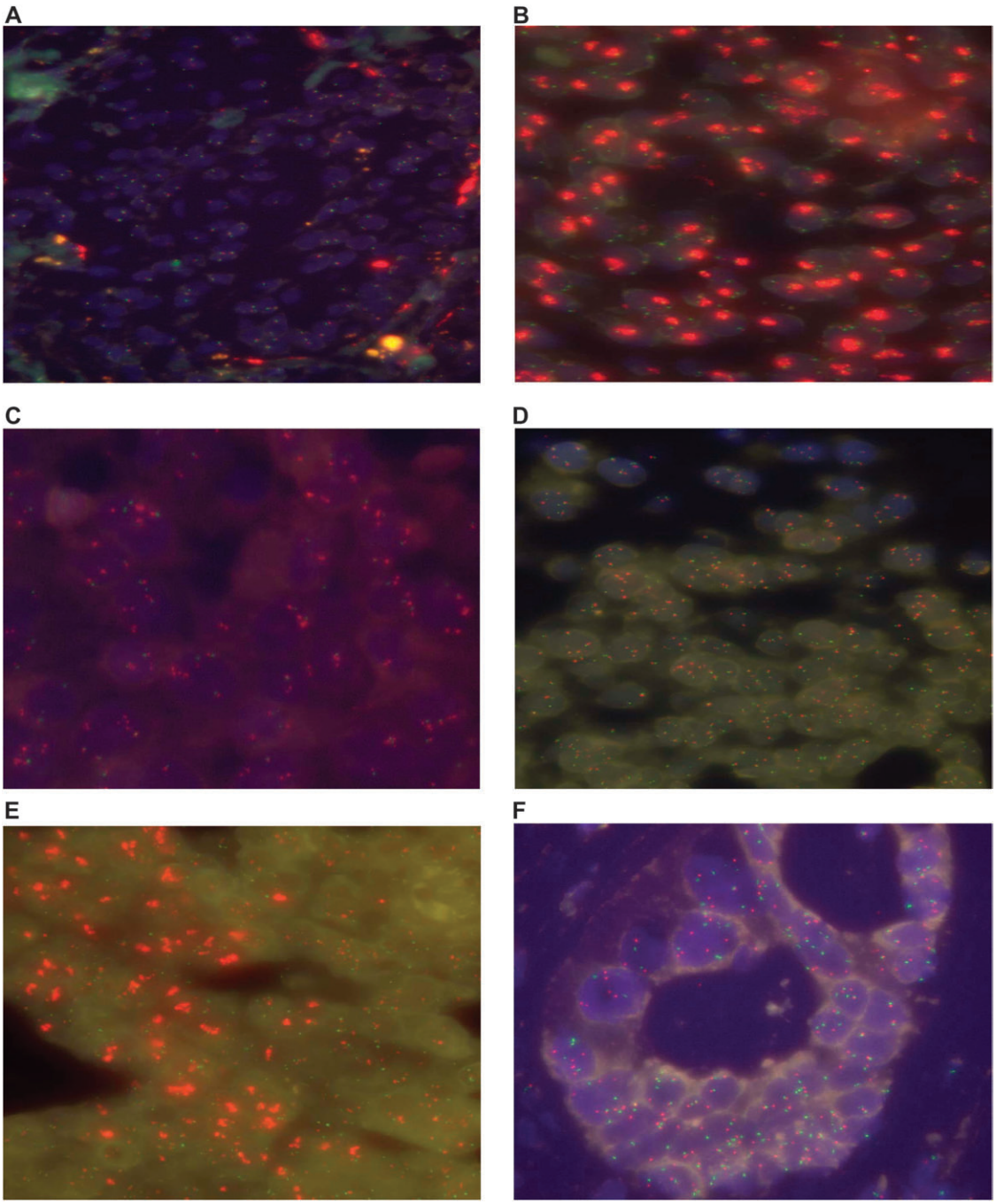

Figure 1. Representative FISH analyses showing tumors comprising cells with (A) normal gene copy number (two signals for each probe per cell); (B) high-level *EGFR* gene amplification, as demonstrated by the presence of large *EGFR* signal clusters; (C) low/moderate-level gene amplification, as demonstrated by the presence of small *EGFR* signal clusters; (D) polysomy, as demonstrated by >2 *EGFR*/CEN-7 signals per cell; (E) heterogeneity for *EGFR* copy number, with only a subpopulation showing high-level gene amplification and (F) heterogeneity for *EGFR* copy number, with certain cells showing polysomy and others, normal copy numbers.

EGFR enrichment models

For each evaluable tumor, a FISH score was determined according to one of the five different enrichment models (Table 1). The distribution of FISH scores was comparable between subgroups of tumor samples from the invasive front and tumor center (data not shown). As expected, the median FISH score in models A, C and E which used more stringent criteria for defining a FISH-positive cell was markedly lower than in models B and D, which used less stringent criteria (supplemental Figure 1, available at *Annals of Oncology* online).

In an initial exploration of the predictive potential of *EGFR* FISH status, scatter plots were constructed for each model for survival time versus the respective FISH score for patients in both study arms (Figure 2A). These plots did not demonstrate

Table 3. Average signal counts following FISH analysis (FISH ITT population)

FISH evaluations	Cetuximab + chemotherapy, $n = 158$	Chemotherapy alone, $n = 154$	FISH ITT population, $n = 312$
CEN-7			
Average numbers of signals/cell, *n*			
Median of all patients (range)	2.3 (1.1–6.2)	2.4 (1.2–5.8)	2.3 (1.1–6.2)
Mean of all patients (SD)	2.5 (0.88)	2.5 (0.82)	2.5 (0.85)
Patients in categories defined by average number of signals/cell, *n* (%)			
1–2	61 (39)	50 (32)	111 (36)
>2–3	57 (36)	61 (40)	118 (38)
>3–4	33 (21)	33 (21)	66 (21)
>4	7 (4)	10 (6)	17 (5)
EGFR			
Average numbers of signals/cell, *n*			
Median of all patients (range)	2.6 (1.1–26.8)	2.8 (1.0–43.2)	2.7 (1.0–43.2)
Mean of all patients (SD)	3.4 (3.26)	4.1 (4.77)	3.7 (4.08)
Patients in categories defined by average number of signals/cell, *n* (%)			
1–2	48 (30)	40 (26)	88 (28)
>2–3	50 (32)	49 (32)	99 (32)
>3–4	36 (23)	35 (23)	71 (23)
>4–5	9 (6)	10 (6)	19 (6)
>5	15 (9)	20 (13)	35 (11)
EGFR/CEN-7 ratio			
Average signal ratio/cell			
Median of all patients (range)	1.0 (0.6–10.7)	1.1 (0.5–20.8)	1.1 (0.5–20.8)
Mean of all patients (SD)	1.5 (1.57)	1.9 (2.57)	1.7 (2.13)
Patients in categories defined by average signal ratio/cell, *n* (%)			
0–1	24 (15)	17 (11)	41 (13)
>1–2	119 (75)	116 (75)	235 (75)
>2	15 (9)	21 (14)	36 (12)
EGFR signal clusters			
Decimal fraction of cells per patient with *EGFR* signal cluster present			
Median of all patients (range)[a]	0 (0–1.0)	0 (0–1.0)	0 (0–1.0)
Mean of all patients (SD)	0.1 (0.24)	0.1 (0.30)	0.1 (0.27)
Patients in categories defined by decimal fraction of cells with clusters[b], *n* (%)			
0	139 (88)	132 (86)	271 (87)
>0 to <0.25	5 (3)	3 (2)	8 (3)
0.25–0.75	5 (3)	3 (2)	8 (3)
>0.75 to <1	8 (5)	9 (6)	17 (5)
1	1 (0.6)	7 (5)	8 (3)

[a]0 = no cluster in any cell; 1 = clusters in every cell.

[b]For example, 0.25 equates to 25% of cells having clusters.

CEN-7, probe for centromeric region of human chromosome 7; ITT, intention to treat; SD, standard deviation.

a visible correlation between *EGFR* FISH score and survival time for any model, in either study arm. The process was repeated for each model for the analysis of PFS time versus the respective FISH score for patients in both study arms (Figure 2B), with similar results. Evaluation of the misclassification error rates further supported the lack of predictive utility of *EGFR* FISH status in relation to overall survival and PFS (see supplemental Results, available at *Annals of Oncology* online). Box plots of FISH score versus best overall response for each model were also constructed. As for the other efficacy end points, these plots did not show a visible correlation between parameters (Figure 2C).

Colorado model

The Colorado model (Table 1) was used to define tumor *EGFR* FISH status for patients in both study arms. Treatment outcome was then assessed according to FISH status. Using this scoring system, 32% of patients were deemed to have *EGFR* FISH-positive tumors. No significant association was apparent for this model between elevated *EGFR* copy number and overall survival, PFS or best overall response (Table 4).

discussion

The collection of tissue samples during the course of large randomized studies in different settings provides a powerful platform to assess the predictive potential of candidate biomarkers, with the analysis of the control arm allowing discrimination between effects, which are prognostic for standard treatment or predictive for the experimental therapy [25]. Evaluation in such individual studies is important as particular mutational or epimutational events which may occur across various tumor types can have different phenotypic consequences in different cell types or against a background of other disease-typical genetic lesions. Consequently, the same mutational event may be predictive for a treatment agent in one tumor type and not another. This has been exemplified in the case of cetuximab by the contrasting data from randomized studies in advanced colorectal cancer and advanced NSCLC, where *KRAS* codon 12/13 mutations are predictive for treatment benefit for cetuximab plus standard chemotherapy compared with chemotherapy alone in the former setting [26, 27] but not the latter [16, 17]. The consequence of such findings is that the potential utility of predictive biomarkers cannot be assumed to be generalizable for a given agent and must be assessed specifically in each tumor type and in each treatment setting. In relation to SCCHN, *KRAS* is mutated (at least in the above-mentioned codons) in only a small fraction of cases [28–30], and therefore, *KRAS* status is not likely to be a useful predictive marker for cetuximab benefit in this disease.

The current study, the largest of its type in this setting, which included an extensive series of tumor samples from 312 patients, represents a truly comprehensive analysis investigating the influence of a disease-relevant candidate biomarker, *EGFR* copy number status, on clinical outcome in patients with R/M SCCHN treated with cetuximab plus platinum-based chemotherapy as part of a large randomized phase III study. As

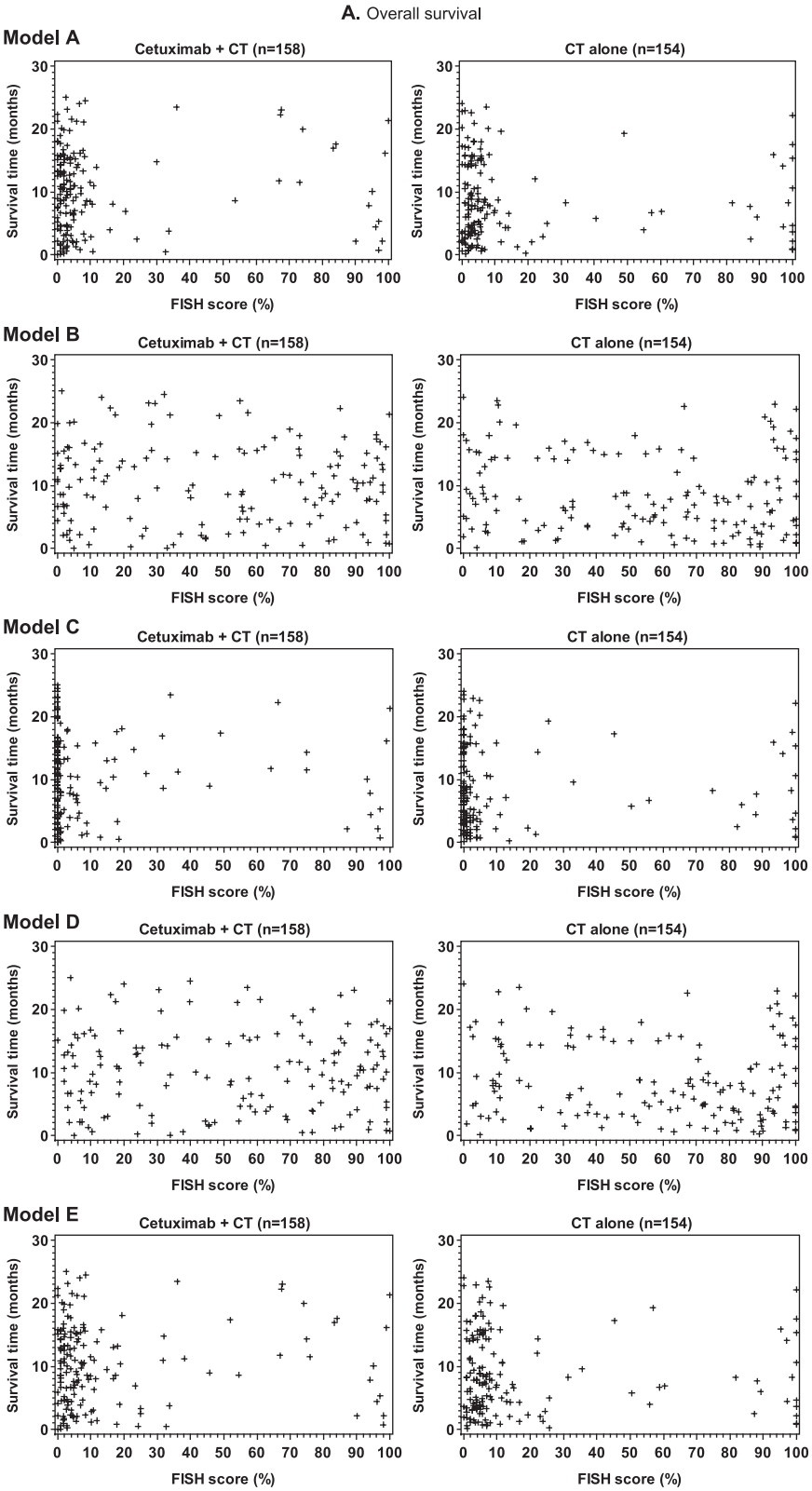

Figure 2. Scatter and box plots did not demonstrate an association between FISH score and (A) overall survival time, (B) progression-free survival (PFS) time or (C) best overall response, for patients in either study arm, when *EGFR* copy number was analyzed according to enrichment models A–E, as indicated. The upper and lower boundaries of each box plot represent the 25th and 75th percentile and the horizontal lines within the box represent the median values. The bars extend to the last observation not defined as an extreme value (represented by + symbols) or to the minimum/maximum values if an extreme value was not identified. CR, complete response; CT, chemotherapy; NE, not evaluable; PD, progressive disease; PR, partial response; SD, stable disease.

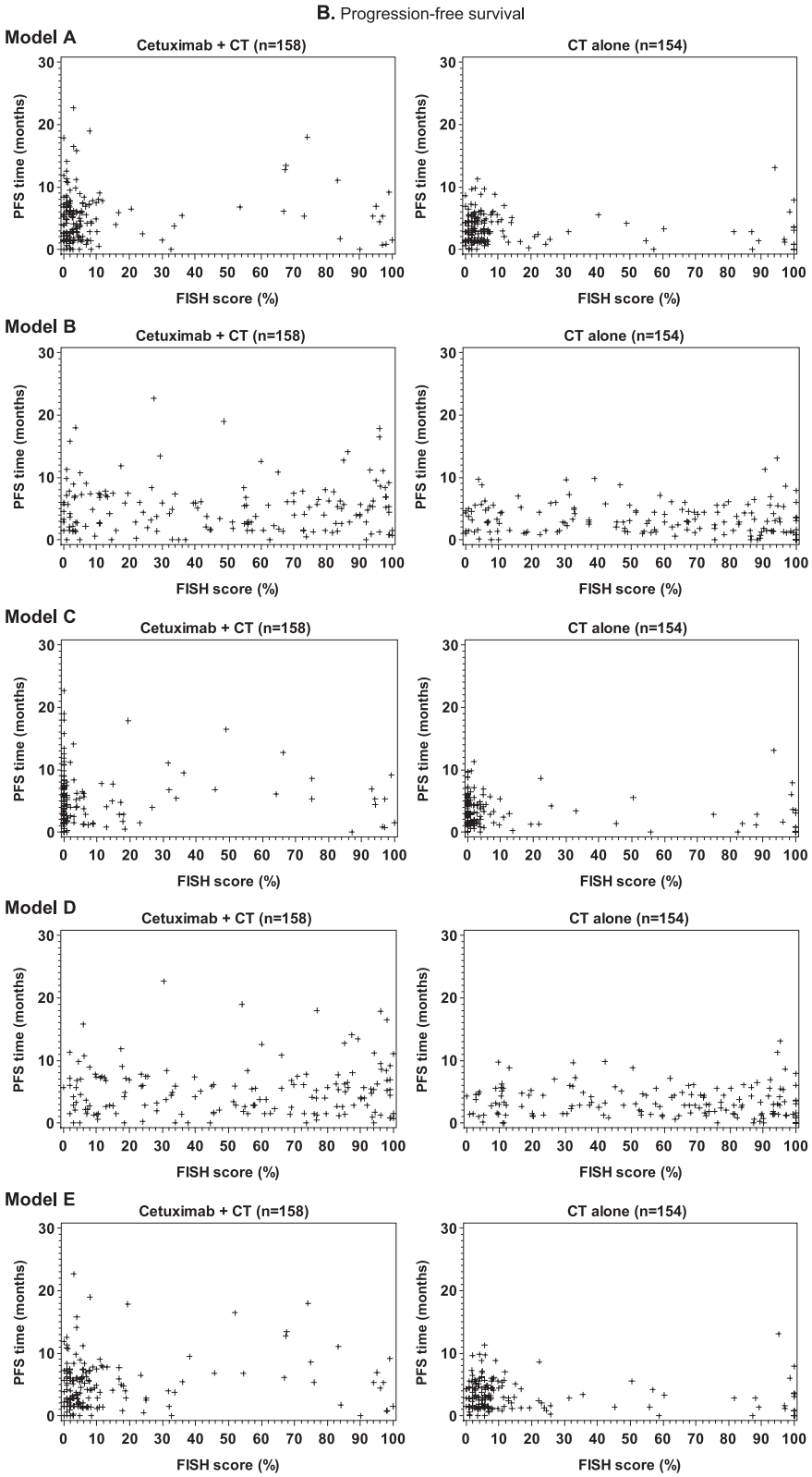

Figure 2. *(Continued)*

the first such exploratory analysis, an appropriate threshold for abnormal copy number on which this and future similar studies could be based had to be determined. By using different enrichment models and calculating a FISH score for each tumor, a broad spectrum of thresholds (from moderate to high) was tested. In addition to these models, the Colorado scoring system, which has been used to demonstrate the predictive utility of *EGFR* gene copy number in NSCLC

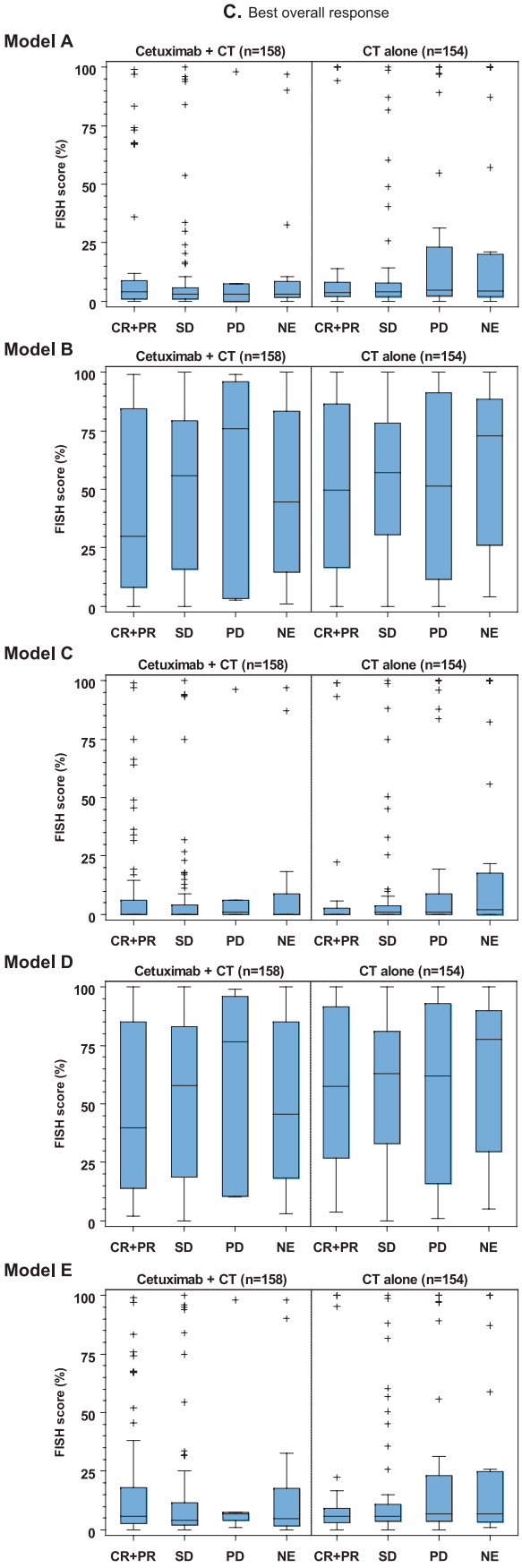

Figure 2. *(Continued)*

Table 4. Colorado FISH status according to tumor site and efficacy according to FISH status (FISH ITT population)

Parameter	Cetuximab + chemotherapy		Chemotherapy alone	
	FISH+, $n = 50$	FISH−, $n = 108$	FISH+, $n = 51$	FISH−, $n = 103$
Overall survival time				
Median, months	10.5	10.6	7.2	7.8
Hazard ratio[a]	1.02		1.04	
(95% CI)	(0.69–1.51)		(0.71–1.51)	
P value	0.93		0.86	
PFS time				
Median, months	6.2	5.7	3.1	4.1
Hazard ratio[a]	0.86		1.05	
(95% CI)	(0.58–1.27)		(0.71–1.54)	
P value	0.46		0.81	
Best overall response rate, %	36.0	34.3	11.8	22.3
Odds ratio[b]	1.08		0.46	
(95% CI)	(0.54–2.18)		(0.18–1.22)	
P value	0.83		0.12	

[a]Hazard ratios <1 correspond to benefit for FISH+ patients.
[b]Odds ratios >1 correspond to benefit for FISH+ patients.
CI, confidence interval; PFS, progression-free survival.

[12], was also evaluated for its predictive potential in this setting.

Considering each of these models covering a range of stringencies, no association of *EGFR* copy number status with overall survival, PFS and best overall response was found. Given the extensive nature of this analysis, it seems reasonable to conclude that *EGFR* copy number status as determined by FISH is not a predictive biomarker for the efficacy of cetuximab combined with platinum/5-FU in the first-line treatment of R/M SCCHN. Although there was a trend for a higher response rate in patients receiving chemotherapy alone with *EGFR* FISH-negative compared with FISH-positive tumors, according to the Colorado scoring system, no robust association between *EGFR* copy number status and any efficacy measure was detected in the overall study population (data not shown). Thus, *EGFR* copy number status does not appear to be a prognostic marker in this setting.

High *EGFR* copy number was previously found to be a marker of poor prognosis in a FISH analysis of a heterogeneous population of 82 patients with SCCHN, 75 of whom were assessable for FISH [22]. Seventy-two primary tumor blocks were initially available from patients who had received no prior anticancer treatment and 14 from patients with recurrent tumors (four paired samples). All patients in the survival analysis were treated with curative intent. The difference between this and the current study in relation to the assessment of the prognostic potential of *EGFR* copy number may be due to the dissimilarity of the patient populations analyzed (R/M SCCHN in the current study versus potentially curable stages I–IV patients in the previous study). Analyzed tissues in the current study were essentially therefore derived from patients with more advanced disease who were to receive palliative treatment. In this context, we cannot derive a definitive

conclusion with respect to patients who might be treated with curative intent with cetuximab since it could well be that *EGFR* copy number has prognostic and/or predictive utility in this setting.

In relation to the mean signal counts, 40% of tumors had *EGFR* copy numbers of >3 and 11% of tumors had copy numbers of >5. The tumor *EGFR*/CEN-7 ratio was >2 for 12% of patients and 11% of patients had *EGFR* signal clusters in ≥25% of their tumor cells. Applying the Colorado system, 32% of tumors were scored as *EGFR* FISH positive. Taken together, these data indicate that a moderate increase in *EGFR* copy number is a common event in SCCHN, with high-level amplification of the gene occurring in a small fraction of tumors (∼11%).

The *EGFR* copy number data in the current study are in the range of values reported from earlier FISH analyses [22, 23, 31, 32]. Two smaller studies using the Colorado scoring system found incidences of FISH-positive tumors of 57% (43 of 75 patients) [22] and 13% (4 of 31 patients) [31], respectively. Analyzing a large series of SCCHN samples using a tissue microarray, Freier et al. [32] reported that 13% (63 of 496) of tumors had 10% of cells showing ≥8 signals or tight signal clusters from the gene-specific probe, which is comparable to the incidence of high-level *EGFR* amplification reported in this study. However, it should be noted that even among patients in the current study whose tumors had high-level increases in *EGFR* gene copy number based on the more stringent enhancement models, no clear distinction in relation to survival benefit was observed (Figure 2A).

In summary, the retrospective analysis of tissue collected during the randomized phase III EXTREME study has indicated that tumor *EGFR* copy number status is not a predictive biomarker for the efficacy of cetuximab plus platinum/5-FU administered as first-line therapy to patients with R/M SCCHN. Therefore, analyzing *EGFR* copy number by FISH in this setting before the administration of cetuximab does not appear to provide any clinically relevant information for the physician. This study has therefore shown that the benefit conferred by the addition of cetuximab to standard chemotherapy for this disease is independent of tumor *EGFR* copy number.

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disclosure

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