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EGFR Expression and Survival in Patients Given Cetuximab and Chemoradiation for Stage III Non-Small Cell Lung Cancer: A Secondary Analysis of RTOG 0324

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Abstract

Purpose—We investigated whether expression of epidermal growth factor receptor (EGFR) was associated with survival and disease control in this secondary analysis of a phase II trial of cetuximab+chemoradiation for stage III non-small cell lung cancer.

Methods—Patients received cetuximab weekly before and during radiation (63 Gy/35 fractions/7 weeks) with weekly carboplatin+paclitaxel. We analyzed EGFR expression by immunohistochemistry (IHC) and fluorescence in situ hybridization (FISH) in pretreatment biopsy specimens and compared findings with overall and progression-free survival (OS, PFS) and time to progression (TTP).

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Results—Specimens for IHC and FISH were collected from 51 and 45 of 87 evaluable patients. Pretreatment characteristics did not differ for patients with (n=51) or without (n=36) EGFR IHC data, or with (n=45) or without (n=42) FISH data. However, patients without IHC data had worse OS (HR=1.63, $P=0.05$), worse PFS (HR=1.88, $P=0.008$), and worse TTP [HR=1.99, $P=0.01$] than those with IHC data. EGFR protein expression was not related to pretreatment characteristics or OS; FISH-positive disease was associated with better performance status but not with OS, PFS, or TTP.

Conclusions—Surprisingly, outcomes differed not by EGFR expression but by the availability of samples for analysis, underscoring the importance of obtaining biopsy samples in such trials.

Keywords

Clinical trials identifier NCT00081302; RTOG 0324; cetuximab; inoperable lung cancer; Erbitux

INTRODUCTION

Lung cancer remains the leading cause of cancer-related death worldwide, with 221,130 new diagnoses and 156,940 deaths in the United States in 2011 [1]. Non-small-cell lung cancer (NSCLC) accounts for approximately 85% of lung cancer diagnoses. For the 35% to 40% of patients who present with locally advanced, inoperable disease, the recommended treatment is thoracic radiation therapy with chemotherapy for patients who can tolerate it [2]. The potential benefit of adding molecularly targeted agents to radiation, with or without chemotherapy, is being actively investigated.

The molecular signaling pathway involving the epidermal growth factor receptor (EGFR) is of particular interest given its association with resistance to both cytotoxic chemotherapy and radiation therapy in cancer cell lines [3,4]; EGFR is also a validated therapeutic target in recurrent or metastatic NSCLC [5]. The anti-EGFR IgG₁ monoclonal antibody cetuximab targets the extracellular domain of the EGFR and binds to the receptor more avidly than the naturally occurring ligand. Preclinical findings suggest that cetuximab can amplify the response to both chemotherapy and radiotherapy [6,7]. Clinical studies of cetuximab with various chemotherapy regimens for metastatic NSCLC have shown that cetuximab is somewhat effective and tolerable and has a manageable safety profile [8–12]. Several phase II trials of cetuximab and radiation, with or without chemotherapy, for stage III NSCLC [13–18] have likewise shown promising but inconclusive results. Phase III evidence of the ability of cetuximab to enhance tumor radiosensitivity and chemosensitivity has been confirmed clinically in two trials: one a randomized phase III trial in patients with head and neck squamous cell carcinoma (HNSCC) given radiotherapy with or without cetuximab [19,20], and the other the First-Line Erbitux in Lung Cancer (FLEX) trial, in which adding cisplatin to a vinorelbine-and-cisplatin regimen improved overall survival (OS) for chemotherapy-naïve patients with metastatic NSCLC [12].

These findings led the Radiation Therapy Oncology Group (RTOG) to undertake RTOG 0324, a phase II trial with the hypothesis that adding an EGFR-targeting agent to chemoradiation would improve the efficacy of that therapy for stage III NSCLC. Findings from this trial, which showed that the combination of cetuximab and chemoradiation is

feasible and has promising activity in terms of OS, are reported elsewhere [18]. The question that remains unanswered, however, is whether EGFR expression status affects disease control or survival in patients receiving cetuximab with radiation, with or without chemotherapy. The purpose of the current study, a secondary objective in RTOG 0324, was to address that question by investigating potential associations between EGFR expression and OS and disease progression after cetuximab and concurrent chemoradiation.

PATIENTS AND METHODS

Details of patient selection, evaluation, and treatment in the multicenter RTOG 0324 trial are described elsewhere [18]. Briefly, patients must have had histologically or cytologically documented stage III NSCLC with good performance status (Zubrod 0–1) and adequate hematologic, hepatic, and renal function. Exclusion criteria included any prior therapy with any other EGFR-targeting agent or a chimerized monoclonal antibody or a history of interstitial pneumonitis, cardiac disease, neuropathy or other cancer (aside from skin cancer or in situ cancer).

Treatment details are also reported elsewhere [18]. Briefly, patients were given cetuximab (400 mg/m²) on day 1 of week 1 and then weekly (250 mg/m²) in weeks 2–17, i.e., through the end of the treatment period. Radiation therapy (63 Gy in 35 fractions over 7 weeks) was begun in week 2, with 6 doses of weekly carboplatin (AUC 2) and paclitaxel (45 mg/m²) followed by carboplatin (AUC 6) and paclitaxel (200 mg/m²) in weeks 12–17. All radiation was to be delivered in standard fractionation schemes as either 2D or 3D conformal radiotherapy. Intensity-modulated radiation therapy was not allowed.

Tumor specimens were requested but not required. Specimens were obtained before treatment via biopsy or fine needle aspiration from the primary tumor and metastatic nodes, if any, embedded in paraffin blocks, and sent to the RTOG Biospecimen Resource before being sent to K. Kian Ang at MD Anderson Cancer Center for immunohistochemical (IHC) staining for EGFR expression or to Fred R. Hirsch at the University of Colorado Cancer Center for fluorescence in situ hybridization (FISH) analysis of EGFR gene copy number as described below.

EGFR immunoassay

Total EGFR protein was stained for IHC analysis as described elsewhere [21]. Interassay consistency was monitored by including positive and negative controls (sections of a high-EGFR-expressing lung cancer specimen stained with primary antibody and an isotype-matched irrelevant antibody) with each batch of staining, and measurements were normalized to these controls (negative control=0, positive control=100%).

EGFR protein expression intensity was measured with Image Pro Plus (v4.5.019; Media Cybernetics, Silver Spring, MD) by investigators without knowledge of the clinical data. Briefly, given the small size of the specimens, five fields were chosen (instead of the nine described previously [21]) at random and the tumor area with the strongest staining was analyzed. The mean optical density (MOD) and staining index (SI) were not weighted to avoid the assumption that bigger tumor fields would better represent the whole tumor.

Images were captured with a 20× objective. Stromal components were removed by interactive cut-and-paste techniques. The resulting area of interest included the whole tumor, stained or non-stained. Parameters measured were MOD, measured over the labeled areas within the tumor, proportional to the mean stain concentration; SI (the proportion of stained area relative to total area of the structures); and quick score ($QS = MOD \times SI/100$).

EGFR FISH analysis

FISH analysis was performed as described elsewhere [22]. Tumors with ≥ 4 copies of the EGFR gene in ≥ 40% of the cells (high polysomy) or tumors with EGFR gene amplification (a gene-to-chromosome ratio ≥ 2 or the presence of gene cluster or ≥ 15 gene copies in ≥ 10% of cells) were considered FISH-positive, and all other tumors were considered FISH-negative. All FISH analyses were performed independently by two investigators blinded to patient clinical characteristics or treatment outcome. FISH analyses were done only on histologic material.

Data analysis

Parameters of EGFR expression were sent to the RTOG Statistical Center for correlative analysis. To determine whether the study population was representative of the whole group, the known clinical prognostic profile and outcome endpoints of patients with successful EGFR assays were first compared with those of patients with no or insufficient specimens. The magnitude and distribution of pretreatment EGFR expression were then assessed using descriptive statistics along with their association with known tumor- and patient-related prognostic variables and recursive partitioning analysis for survival and local-regional (LR) failure. Finally, the magnitude and distribution of pretreatment EGFR expression were correlated with OS, progression-free survival (PFS), and pattern of relapse (LR recurrence and distant metastasis [DM]).

Statistical methods

Categorical comparisons of baseline characteristics were done with Pearson chi-square tests or Fisher exact tests; continuous baseline data were compared with *t* tests. OS and PFS were estimated using the Kaplan-Meier method and compared with log-rank tests. Times to failure and DM were estimated by the cumulative incidence method and tested with Gray's test. Multivariate analysis was done with the Cox proportional hazards model.

RESULTS

RTOG 0324 opened to accrual March 8, 2004, and closed June 3, 2005 after accruing 93 patients, 87 of whom were ultimately evaluable for analysis. A total of 40 institutions registered the 87 cases analyzed here; 18 of those institutions (45%) registered only 1 case. Fifty-one (59%) of the evaluable patients had provided tissue samples that could be evaluated for EGFR protein expression; 45 patients (52%) had tissue available for FISH analysis; and 42 patients were evaluated by both IHC and FISH. The median EGFR IHC QS was 0.652 (range 0.0–0.99).

Pretreatment characteristics did not differ between patients who had EGFR data (n=51) and those who did not, either because tissues were not sent (n=25) or were not evaluable for EGFR IHC (n=6) or FISH (n=17) (Table 1). Those with EGFR data may have been more likely to have squamous tumors ($P=0.065$ for IHC and $P=0.069$ for FISH).

However, patients whose tumors could not be evaluated for EGFR IHC fared worse in terms of OS (higher risk of death) [HR=1.63 (95% confidence interval {CI} 0.99–2.67), $P=0.050$], PFS (higher risk of progression or death) [HR=1.88 (95% CI 1.18–3.01), $P=0.008$], and time to progression (higher risk of progression) [HR=1.99 (95% CI 1.17–3.38), $P=0.01$]. The median OS time for patients with EGFR data was 30.4 months (95% CI 20.6–39.8) versus 12.8 months (95% CI 6.9–19.2) for patients without EGFR data ($P = 0.048$). The median PFS time for patients with EGFR data was 10.8 months (95% CI 8.5–28.2) versus 7.2 months (95% CI 4.2–9.2) for those without EGFR data ($P=0.007$). The 36-month progression rate for patients with EGFR data was 57.3% (95% CI 43.3–71.3) versus 70.6% (95% CI 54.5–86.6) for patients without EGFR data $P = 0.07$ (Fig. 1). Thus in terms of survival and disease progression, the patients for whom EGFR protein expression was evaluable seemed to have more favorable prognosis. No difference was found in OS, PFS, or time to progression according to whether samples were available for FISH (n=45) or not (n=42) (not shown).

No difference in pretreatment characteristics or in OS was noted according to EGFR QS above (n=25) versus below the median (n=26) [HR=1.43 (0.75–2.74), $P=0.28$] (Table 2). Similarly, no association was found between EGFR QS and OS when QS was analyzed as a continuous variable (not shown). The median OS time for patients with QS ≤ 0.652 was 32.0 months (95% CI 22.7–not reached) versus 29.2 months (95% CI 11.3–41.8) for those with QS >0.652 ($P = 0.27$). The corresponding median PFS times were 12.3 months (95% CI 8.5–49.7) for QS ≤ 0.652 vs. 10.4 months (95% CI 7.7–28.2) for QS >0.652 ($P=0.15$) (Fig. 2). Similarly, no association was found between OS (univariate $P=0.99$; multivariate $P=0.33$) or PFS (univariate $P=0.65$; multivariate $P=0.34$) for patients with FISH+ disease versus those with FISH– disease. Moreover, no correlations were found between patients who had high EGFR expression by IHC and those with FISH+ disease (low IHC/FISH– = 26%; low IHC/FISH+ = 17%; high IHC/FISH– = 24%; high IHC/FISH+ = 33%). However, this analysis was not powered to detect such an association.

Regarding patterns of failure, 10 patients in the EGFR QS ≤ 0.652 group (38.5%) had no progression versus 7 of those with QS >0.652 (28.0%). Of the 34 patients who did experience disease failure, that failure was local only in 7 (44%) of the patients with EGFR QS ≤ 0.652 and local only in 6 (33%) in patients with EGFR QS >0.652 ($P=0.73$); another 2 patients in the first group and another 5 in the second had DM in addition to local failure (9 [57%] vs. 11 [61%]). Failure was metastatic only in 5 (31%) of the patients with EGFR QS ≤ 0.652 and in 6 (33%) of the patients with EGFR QS >0.652 ($P=1.00$).

Finally, analysis of OS and PFS by histology (squamous vs. nonsquamous) with Cox proportional hazards models revealed no differences in this population (not shown).

DISCUSSION

In this secondary analysis of RTOG 0324, we sought to correlate outcomes with the expression of EGFR in samples obtained before the initiation of concurrent chemoradiation therapy with cetuximab for stage III NSCLC. We found no differences in OS or time to progression according to EGFR expression level for patients treated with carboplatin, paclitaxel, radiation, and cetuximab. However, this analysis was not powered to detect such differences. Indeed, our discovery that outcomes were worse among patients for whom samples were not available for IHC further hinders our ability to draw firm conclusions based on our findings.

The question of whether adding cetuximab to either radiotherapy, chemotherapy, or both is beneficial for HNSCC and in NSCLC remains in debate. For example, two recent trial reports showed no improvement in outcome from the addition of cetuximab to radiation and cisplatin for locally advanced HNSCC [23] or locally advanced NSCLC [24]. On the other hand, a phase III trial reported by Bonner et al. [19,20] showed improvements in both local control and OS from adding cetuximab to radiotherapy for HNSCC. Another study that did not involve radiation, the FLEX trial, found that adding cetuximab to platinum-based chemotherapy was beneficial for patients with EGFR-expressing metastatic NSCLC [12]. With regard to analyses of biomarkers, the picture is less clear. One subsequent analysis using specimens from the FLEX trial [25] showed that KRAS and EGFR mutation status, EGFR copy number, and PTEN expression did not predict treatment outcome; however, another analysis of the same trial [26] revealed that high tumor EGFR expression, independent of tumor histology, identified patients with advanced NSCLC who were most likely to benefit from the addition of cetuximab to platinum-based first-line chemotherapy. No corresponding survival benefit was found from adding cetuximab to chemotherapy in a group with low EGFR expression. Those investigators interpreted their findings as supporting the biological premise that high levels of EGFR expression in NSCLC are likely to indicate tumor dependency on EGFR signaling and thus sensitivity to cetuximab therapy, a premise supported by others as well [27]. However, in a phase III trial designed in support of FLEX (the Bristol-Myers-Squibb trial BMS099), another group [28] found that increased EGFR expression and EGFR amplification did not predict the efficacy of cetuximab in combination with taxane-carboplatin chemotherapy for patients with advanced NSCLC. To our knowledge, only two other studies have been published in which EGFR expression by IHC was evaluated in relation to survival in patients who received cetuximab and radiation [29,24]. An exploratory analysis in the SWOG S0429 pilot study revealed that neither membrane nor cytoplasmic staining for EGFR correlated with OS or PFS in the 11 patients tested. However, that study had not been designed to measure survival as the primary endpoint, which with the small number of patients hampered interpretation of these findings. A subset of findings from another trial [24] suggested that the addition of cetuximab to cisplatin-based chemoradiation did not affect OS or disease control in 17 patients known to have high EGFR expression. Unfortunately the current study was also unable to resolve the open question as to whether benefit may be related to expression of EGFR by the tumor in NSCLC.

Our results from this admittedly small study differ from those of RTOG 9003, a large phase III trial testing the efficacy of various altered fractionation regimens for patients with advanced HNSCC [30]. In a correlative biomarker analysis from that trial [21], EGFR expression did not correlate with disease stage at presentation or other known clinical prognostic variables, but EGFR expression was a strong prognostic indicator for OS and DFS and was highly predictive of LR relapse but not DM.

The differences between our results and those from the head and neck trials [20,30] have several possible explanations, including differences in tumor histology (our NSCLC patients had SCC, adenocarcinoma, or undifferentiated large cell carcinoma but the head and neck trial included only patients with SCC), treatment regimens (concurrent chemoradiation and cetuximab in our study vs. radiation alone in the head and neck trial), and numbers of patients (51 in our study vs. 155 in one study [30] and 424 in the other [20]).

Our study did have several shortcomings, chief among them the small numbers of specimens analyzed and the fact that the trial was a phase II study rather than a large prospective phase III trial. Another shortcoming was our inability to link tumor histology (squamous vs. non-squamous) with tumor location (central vs. peripheral). One could speculate that cases that could be analyzed by IHC or FISH were more likely to have been squamous, more likely to be centrally located [31] (i.e., sufficiently close to the main bronchus to allow biopsy via bronchoscopy as opposed to fine-needle aspiration of more peripheral lesions), or both. Another possibility is that some tumors that could not be analyzed by IHC may have been more rapidly progressive, which would not have affected disease staging but may have contributed to worse prognosis. Another intriguing possibility is that the unexpected correlation between treatment outcome and availability of biopsy material could be a treatment-center effect, in that the 22 centers that were motivated to participate in the translational part of this trial by providing tissue samples may also have provided better treatment. Unfortunately, these possibilities cannot be assessed with the current dataset.

Nevertheless, strengths of this study include the IHC staining and analysis of all specimens by a single laboratory with considerable experience in IHC analysis, and the FISH analyses by another such laboratory. The expression of EGFR protein is often, but not always [32], associated with polysomy or amplification of the EGFR gene [33]. The lack of statistical correlation between EGFR IHC and EGFR copy number in the current study could reflect several factors, including the small number of patients, small size of biopsy samples, and possibly differences in the antibodies and techniques used for IHC analysis over the course of the study. Some evidence from preclinical models of HNSCC suggests that FISH may be a more reliable biomarker of local tumor control than EGFR protein expression [32], as the latter may change over the course of irradiation. However, many studies have demonstrated that EGFR protein expression and EGFR gene copy number are independent predictors for response to EGFR tyrosine-kinase inhibitors [34,35].

Although the lack of correlation found in the current analysis was disappointing, the success of the treatment regimen, which produced the best clinical results to date for stage III NSCLC (median survival time 23 months, 2-year survival rate 49.5%, and 8% grade 3–4 pneumonitis and esophagitis) led to the amendment of the phase III trial RTOG 0617, the

original purpose of which was to compare 60 Gy in 30 fractions vs. 74 Gy in 37 fractions with concurrent carboplatin and paclitaxel for locally advanced NSCLC, to incorporate a comparison of chemoradiation with or without cetuximab. Given the larger size and prospective nature of RTOG 0617, analyses of specimens from that trial for EGFR expression and other potential biomarkers may well reveal biomarkers that do correlate with outcome. Preliminary results from this trial presented at the 15th World Conference on Lung Cancer in Sydney, Australia in October 2013 indicated that the addition of cetuximab to chemoradiation therapy had no effect on OS or PFS [36]. Nevertheless, the patients in that trial were not selected on the basis of EGFR expression, cellular localization (membrane vs. nucleus [27]) or mutation status, and it remains to be seen whether these characteristics could help to identify patients who are more likely to benefit from the addition of cetuximab or other EGFR inhibitors to chemoradiotherapy.

In conclusion, in this preliminary correlative analysis of findings from RTOG 0324, we found no differences in OS or time to failure by EGFR expression, but this study was not powered to detect such differences, nor can such conclusions be supported given the bias in favor of patients from whom samples could be collected. Also, biopsy specimens were requested but not required in RTOG 0324; future prospective trials should include a requirement for core needle biopsy in all subjects, to be obtained with the assistance of interventional radiologists as needed. If correlative analyses from the larger prospective RTOG 0617 trial reveal differences in survival or progression related to EGFR expression, then quantifying EGFR expression might be valuable for selecting patients for various available therapy modalities or for enrollment in trials of novel therapies targeting EGFR or its downstream signaling pathways.

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Figure 1.

Overall survival (A), progression-free survival (B), and time to progression (C) curves for patients with or without information on EGFR expression.

Figure 2.
Overall survival (A), progression-free survival (B), and time to progression (C) curves according to EGFR quick score, dichotomized at the median 0.652.

Table 1

Pretreatment Characteristics by Availability of Tissue Samples for EGFR Analysis

	No Samples For EGFR IHC (n=36)	Samples for EGFR IHC (n=51)	No Samples for EGFR FISH (n=42)	Samples for EGFR FISH (n=45)	P Value*	P Value*
Age, years						
Median	64	64	63.5	65	0.746	0.091
Range	46–78	42–85	42–78	44–85		
FEV1, liters						
Median	2.12	1.90	2.10	1.90	0.562	0.435
Range	1.23–4.26	1.26–4.20	1.23–4.26	1.26–4.2		
FEV1, % of predicted value						
Median	73	73	73	73.5	0.959	0.745
Range	45–113	40–133	43–113	40–133		
Sex	n (%)	n (%)	n (%)	n (%)	P Value*	P Value*
Male	21 (58)	30 (50)	24 (57)	27 (60)	0.964	0.79
Female	15 (42)	21 (41)	18 (43)	18 (40)		
Race						
Asian	0	1 (2)	0 (0)	1 (2)		
Black or African American	3 (8)	7 (14)	4 (10)	6 (13)		
White	33 (92)	43 (84)	38 (90)	38 (84)		
Non-White	3 (8)	8 (16)	4 (10)	7 (16)	0.351 [†]	0.40 [‡]
White	33 (92)	43 (84)	38 (90)	38 (84)		
Ethnicity						
Hispanic	1 (3)	0	1 (2)	0 (0)		
Not Hispanic	35 (97)	49 (96)	41 (98)	43 (96)		
Unknown	0	2 (4)	0 (0)	2 (4)		
Zubrod score						
0	16 (44)	25 (49)	24 (57)	17 (38)	0.674	0.071
1	20 (56)	26 (51)	18 (43)	28 (62)		

Disease stage	No Samples For EGFR IHC (n=36)	Samples for EGFR IHC (n=51)	No Samples for EGFR FISH (n=42)	Samples for EGFR FISH (n=45)	P Value*	P Value*
IIIA	18 (50)	22 (43)	19 (45)	21 (47)	0.527	0.89
IIIB	18 (50)	29 (57)	23 (55)	24 (53)		
Tumor histology						
Non-squamous	28 (78)	30 (59)	32 (76)	26 (58)	0.065	0.069
Squamous	8 (22)	21 (41)	10 (24)	19 (42)		
Lobar location						
Not lower or multiple lobes	29 (81)	41 (80)	32 (76)	38 (84)	0.985	0.33
Lower or multiple lobes	7 (19)	10 (20)	10 (24)	7 (16)		

* χ^2 test for continuous data, Chi-square test for categorical data unless otherwise noted

[†] Fisher's exact test

Table 2

Pretreatment Characteristics by EGFR Protein Expression or Gene Copy Number

	Low Protein QS 0.652 (n=26)	High Protein QS >0.652 (n=25)	P Value *	FISH-Negative (1-4 copies) [†] (n=24)	FISH-Positive (5-6 copies) [†] (n=21)	P Value *
Age						
Median	63	66	0.557	62	68	0.548
Range	42-84	44-85		(44-84)	(44-85)	
	n (%)	n (%)	P Value *	n (%)	n (%)	P Value *
Sex						
Male	12 (46)	18 (72)	0.061	13 (54)	14 (67)	0.39
Female	14 (54)	7 (28)		11 (46)	7 (33)	
Race						
Asian	1 (4)	0		1 (4)	0 (0)	
Black or African American	3 (12)	4 (16)		5 (21)	1 (5)	
White	22 (85)	21 (84)		18 (75)	20 (95)	
Non-White	4 (16)	4 (16)		6 (25)	1 (5)	0.10 [‡]
White	22 (85)	21 (84)		18 (75)	20 (95)	
Ethnicity						
Not Hispanic	26 (100)	23 (92)		24 (100)	19 (90)	0.21 [‡]
Unknown	0	2 (8)		0 (0)	2 (10)	
Zubrod score						
0	14 (54)	11 (44)	0.482	5 (21)	12 (57)	0.012
1	12 (46)	14 (56)		19 (79)	9 (43)	
Disease stage						
IIIA	8 (31)	14 (56)	0.069	11 (46)	10 (48)	0.90
IIIB	18 (69)	11 (44)		13 (54)	11 (52)	
Histology						
Non-Squamous	16 (62)	14 (56)	0.688	15 (63)	11 (52)	0.49
Squamous	10 (38)	11 (44)		9 (38)	10 (48)	
Lobar location						

	Low Protein QS 0.652 (n=26)	High Protein QS >0.652 (n=25)	FISH-Negative (1-4 copies) [†] (n=24)	FISH-Positive (5-6 copies) [†] (n=21)	P Value *
Not lower or multiple lobes	22 (85)	19 (76)	19 (79)	19 (90)	0.42 [‡]
Lower or multiple lobes	4 (15)	6 (24)	5 (21)	2 (10)	

* P values were generated by t tests for continuous data and χ^2 tests for categorical data unless otherwise noted

[†]Tumors with 4 copies of the EGFR gene in 40% of the cells (high polysomy) or with EGFR gene amplification (a gene-to-chromosome ratio 2 or the presence of gene cluster or 15 gene copies in 10% of cells) were considered FISH-positive; all others were considered FISH-negative.

[‡]Fisher's exact test

Abbreviations: QS, quick score (defined as [mean optical density $\times$ staining index/100]; FISH, fluorescence in situ hybridization