Epidermal Growth Factor Receptor-Related Tumor Markers and Clinical Outcomes with Erlotinib in Non-small Cell Lung Cancer

An Analysis of Patients from German Centers in the TRUST Study

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Introduction: Relationships between clinical outcomes and epidermal growth factor receptor (EGFR)-related tumor markers were investigated in patients with advanced non-small cell lung cancer.

Methods: Patients with stage IIIB/IV non-small cell lung cancer (0–2 prior regimens) received erlotinib (150 mg PO per day). Response and survival were evaluated, and tumor samples were assessed by immunohistochemistry (EGFR, phosphorylated mitogen-activated protein kinase, and phosphorylated AKT protein expression), fluorescence in situ hybridization (FISH; *EGFR* gene copy number), and DNA sequencing (*EGFR*, *KRAS* gene mutations).

Results: Among 311 patients, 8% had a complete/partial response; the disease control rate was 66%. Median Overall survival (OS) was 6.1 months; 1-year survival rate was 27.2%. Two of 4 patients with *EGFR* mutations had tumor responses, versus 2/68 with wild-type EGFR ($p = 0.014$). Progression-free survival (PFS) (HR = 0.31) and OS (HR = 0.33) were significantly prolonged in patients with *EGFR* mutations. Response rate was significantly higher in patients with EGFR FISH-positive (17%) than FISH-negative tumors (6%), and both PFS (HR = 0.58) and OS (HR = 0.63) significantly favored patients with EGFR FISH-positive tumors; median OS was 8.6 months in the EGFR FISH-positive group. None of 17 patients with a *KRAS* mutation had a tumor response, but the impact of *KRAS* mutation status on survival outcomes was of borderline statistical significance. Neither phosphorylated mitogen-activated protein kinase nor phosphorylated AKT immunohistochemistry status had a significant effect on PFS and OS with erlotinib.

Conclusions: The presence of *EGFR* mutations and EGFR FISH-positive tumors may predispose patients to achieving better outcomes on erlotinib, but may have a beneficial impact on prognosis (irrespective of treatment). Prospective, placebo-controlled studies are needed to determine the predictive value of the putative biomarkers.

Key Words: Erlotinib, Non-small cell lung cancer, Epidermal growth factor receptor (EGFR), *EGFR* mutations, *EGFR* gene copy number.

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Intracellular signaling activated by the epidermal growth factor receptor (EGFR) plays a role in tumor growth and progression in many cancers[1,2] and EGFR overexpression occurs in up to 80% of non-small cell lung cancers (NSCLC).[3,4] Erlotinib is a selective inhibitor of EGFR tyrosine kinase activity,[5] demonstrated to significantly prolong survival versus placebo in patients with relapsed NSCLC.[6]

There is currently much interest in determining which patients benefit most from EGFR tyrosine kinase inhibitor (TKI) therapy, and identifying tumor markers that predict clinical outcomes. The findings to date are inconclusive, due to the small number of samples analyzed, and the retrospective nature of most studies.[7,8] One of the largest controlled datasets on potential biomarkers for erlotinib comes from the BR.21 trial.[9–11] While there were trends in the data, multivariate analyses suggested that survival was not significantly influenced by EGFR expression, the number of *EGFR* gene copies, or the presence of *EGFR* mutations.

This paper describes analyses of relationships between clinical benefits and putative tumor markers measured in samples from patients participating in Tarceva Lung Cancer Survival Treatment (TRUST), a large, open-label trial of

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Disclosure: Dr. Heigener was paid honoraria and travel expenses for a presentation on Tarceva on the German Cancer Congress 2008. Dr. Reck was paid travel expenses and honoraria to speak about Erlotinib at several national and international meetings. The other authors declare no conflicts of interest.

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erlotinib (7043 patients in 52 countries). The analysis was limited to patients in German centers. The markers examined were EGFR expression; *EGFR* gene copy number; *EGFR* mutations; *KRAS* mutations; and expression of phosphorylated AKT (pAKT) and phosphorylated mitogen-activated protein kinase (pMAPK). The second objective was to evaluate relationships between different markers.

PATIENTS AND METHODS

Trial Design

The trial is an open-label single-arm study of erlotinib in patients with advanced (inoperable stage III B or IV) NSCLC who failed or were not medically suitable for standard first-line chemotherapy. This analysis relates to patients registered in Germany, who received at least one dose of erlotinib, and for whom a tumor sample was available.

Patients

Patients were eligible if they met the following criteria: ≥18 years with histologically or cytologically confirmed, unresectable, stage III B/IV NSCLC; Eastern Cooperative Oncology Group performance status 0–3; 1/2 prior courses of standard chemotherapy or radiotherapy, or were unsuitable for such treatment; at least 3 to 4 weeks since last treatment (surgery within 4 weeks allowed, if fully recovered); full recovery from toxicities due to prior therapy; adequate hematological, renal, and hepatic function; life expectancy ≥12 weeks; negative pregnancy test for women of child-bearing potential.

Notable exclusion criteria were: evidence of unstable systemic disease; prior treatment with anti-EGFR agents; previous malignancies (last 5 years, other than successful treatment for cervical carcinoma/skin cancer); untreated brain metastases or spinal cord compression; significant ophthalmologic abnormalities (e.g., severe dry eye syndrome, keratoconjunctivitis sicca, Sjögren syndrome, severe exposure keratitis).

Informed consent was obtained, and local or regional ethical committee approval was received by all centers. The trial was conducted in accordance with the Declaration of Helsinki and its subsequent amendments, and with Good Clinical Practice guidelines.

Drug Administration

All patients received erlotinib 150 mg PO per day. (Tarceva; F. Hoffmann-La Roche, Basel, Switzerland). Treatment continued until unacceptable toxicity, disease progression or death, or withdrawal. Dose escalation was not permitted. Dose interruption/reduction was permitted for treatment-related adverse events. Reescalation was not permitted, except after erlotinib-related rash.

Clinical Assessments

Tumor response was assessed using Response Evaluation Criteria in Solid Tumors,[12] at least every 2 months. Responses were confirmed after 4 weeks. Overall survival (OS) and progression-free survival (PFS) were calculated from date of randomization to date of death/date of disease progression or death, respectively. Clinical assessments were at baseline and then every 4 weeks.

Biomarker Analyses

Tumor samples were collected at initial diagnosis or before treatment (sites were asked to provide formalin-fixed, paraffin-embedded tissue blocks or ≥10 unstained slides). Availability of a tissue sample was not mandatory; patients' written consent was required for use of tumor samples. Gene sequencing was performed at the Roche Center for Medical Genomics (Basel, Switzerland), other histopathological and molecular analyses at TARGOS Molecular Pathology GmbH, Kassel, Germany, using technically validated, optimized and standardized assays.

EGFR and KRAS Mutation Analyses

Manual microdissection or laser capture microdissection was performed on formalin-fixed paraffin-embedded tissue sections (to harvest ≥5000 tumor cells and increase sample DNA content). DNA lysates were prepared from microdissected tissue. Amplifications of *EGFR* exons 18–21 and *KRAS* exon 2 and 3 were performed using nested primers.[13] All polymerase chain reactions (PCRs) were performed with HotStarTaq (Qiagen); 95°C × 15 minutes; 95°C × 1 minute, 60°C × 30 seconds, 72°C × 30 seconds, for 30 cycles, then 72°C for 5 minutes, 20 μL reactions. Templates were treated with uracil-N-glycosylase to prevent artifacts. PCR products were purified with PCR96 Cleanup Plates (Millipore). Sequencing reactions were performed using Applied Biosystems Version 3.1 Big Dye Terminator chemistry, and analyzed on an Applied Biosystems 3730 Sequencer. PCR products were sequenced in sense and antisense directions. Only the most common *EGFR* gene mutations were evaluated (E746-A750 deletion in exon 19; L858R in exon 21). Mutations in codons 12 and 13 of exon 2, and codon 61 of exon 3 of *KRAS* were also analyzed. Samples were classed as 'mutated' (≥1 mutation detected), 'wild-type' (no mutations detected), or 'indeterminate' (failed analysis in ≥1 locus and 'wild-type' in the other locus).

Immunohistochemistry and Fluorescence In Situ Hybridization Analyses

EGFR protein expression was assessed using the EGFR PharmDx immunohistochemistry (IHC) kit (#K1492, Dako, Glostrup, Denmark). Samples were rated EGFR-positive if membranous staining was observed in either ≥10% of tumor cells or in any tumor cells ('any staining' cutoff). pMAPK and pAKT were assessed using IHC with the 'Phospho-p44/42 MAPK' antibody (#4376, rabbit clone 2OG11, Cell Signaling, Danvers) and the rabbit monoclonal 'phospho-AKT (Ser473)' antibody (#3787, clone 736E11, Cell Signaling, Danvers), respectively. Cytoplasmic staining of pMAPK and nuclear staining of pAKT were quantified using H-scores calculated from staining intensity (0–3+) and percentage of stained cells (0–100%). The cutoff for positivity was H-score ≥200. *EGFR* gene copy number was assessed by fluorescence in situ hybridization (FISH) using commercial probes (LSI *EGFR* SpectrumOrange/CEP 7 SpectrumGreen Probe [#32–191053], Abbott/Vysis, Des Plaines). Samples with

high gene copy number (high polysomy/gene amplification) were classed as FISH-positive.[14]

Statistics

For binary variables, values were set to 0 (female, squamous-cell carcinoma, never-smoker, IHC/FISH-negative) or 1 (male, adenocarcinoma, current/former smoker, positive IHC/FISH status). For response-based clinical outcomes, ordinal values set were: 0 = progressive disease; 1 = stable disease (SD) <120 days; 2 = SD 120 to 180 days; 3 = SD >180 days; 4 = partial response (PR); 5 = complete response (CR). FISH strata (1–6) were considered ordinal variables and IHC H-scores were considered metric variables. Positive/negative test status is inherently binary.

Differences in response rates and PFS/OS according to biomarker status were tested with Fisher's exact test and the log-rank test, respectively. Associations between variables were assessed using nonparametric tests: Spearman's rank correlation for metric or ordinal variables; Wilcoxon rank sum test for metric/ordinal versus binary variables (the relevant Mann-Whitney [MW] score indicating the strength of association); Fisher's exact test for binary variables (the Phi-coefficient indicating the strength of association).

RESULTS

Patients

The intent-to-treat population (n = 393) comprised all registered patients from Germany with a tumor tissue sample submitted for analysis, who received at least one dose of erlotinib, for whom clinical data was available by the cutoff date (June 21, 2007). Table 1 summarizes the demographic and baseline clinical/tumor characteristics. The majority of patients (79%) had stage IV NSCLC; 77% received erlotinib as second- or third-line therapy.

Efficacy

Clinical information was not available for all patients. Tumor response was available for 311 of 393 patients (17 not evaluable; 65 no data). The overall response rate was 7.9% (26 of 328); 4 CRs and 22 PRs. Fifty-eight percent (189 of 328) had SD. The disease control rate (CR + PR + SD) was 66%. Survival data were available for 392 of 393 patients. Median PFS was 2.33 months (95% confidence interval [CI], 2.00–2.96). Median OS was 6.11 months (95% CI, 5.09–7.29: Figure 1); 1-year survival was 26.2% (95% CI, 21.6–30.7).

Relationships Between Biomarker Status and Clinical Outcomes with Erlotinib

Not all biomarker measurements were available for every patient (Table 1), due to inadequacy/poor quality of tissue and assay failures. Table 2 shows response and survival outcomes on erlotinib according to marker status. Two of 4 patients with *EGFR* mutations had tumor responses (50%) versus 2 of 68 with wild-type *EGFR* (3%; p = 0.014). Response rates were significantly higher in patients with *EGFR* FISH-positive (17%) than FISH-negative tumors (6%), and those with pAKT IHC-positive (21%) than IHC-negative tumors (5%).

Using the positivity criterion for EGFR IHC of ≥10% membranous staining, PFS and OS both favored patients with EGFR IHC-positive tumors (not shown), but neither difference was statistically significant (Table 2). Using the 'any-staining' criterion the differences in PFS and OS were more marked, but did not reach statistical significance.

PFS and OS both significantly favored patients with *EGFR* FISH-positive tumors (Table 2 and Figure 2). Median OS was 8.6 and 6.1 months for the FISH-positive and FISH-negative groups, respectively. *EGFR* FISH status was significantly correlated with response-based clinical out-

TABLE 1. Demographic and Baseline Clinical Characteristics[a] (n = 393)

		n	%
Age	Years: median (range)	65 (31–90)	
Gender	Male/female	232/161	59/41
Ethnic origin	Caucasian/oriental/no data	389/3/1	99/1<1
Histology	Adenocarcinoma/squamous cell carcinoma/other	200/124/69	51/32/18
Smoking status	Never-smoker/former or current smoker/no data	96/296/1	24/75/<1
ECOG PS	0/1/2/3	88/200/84/21	22/51/21/5
Stage	IIIB/IV	81/312	21/79
Line of therapy	1st/2nd/3rd/other/no data	75/158/147/12/1	19/40/37/3/<1
Tumor characteristics			% positive
EGFR IHC (≥10%)	Positive tests/total tests	236/293	81
EGFR IHC (any staining)	Positive tests/total tests	257/293	88
EGFR FISH	Positive tests/total tests	49/208	24
EGFR mutations	Mutation/wild-type/indeterminate/total tests	6/86/103/195	7[b]
KRAS mutations	Mutation/wild-type/indeterminate/total tests	17/97/81/195	15[b]
pMAPK IHC	Positive tests/total tests	48/195	25
pAKT IHC	Positive tests/total tests	54/192	28

[a] For patients with biomarker data.
[b] % mutation rate = [mutations/(mutations + wild-type)] × 100.
ECOG PS, Eastern Cooperative Oncology Group Performance Status; EGFR, epidermal growth factor receptor; IHC, immunohistochemistry; FISH, fluorescence in situ hybridization; pMAPK, phosphorylated mitogen-activated protein kinase; pAKT, phosphorylated AKT.

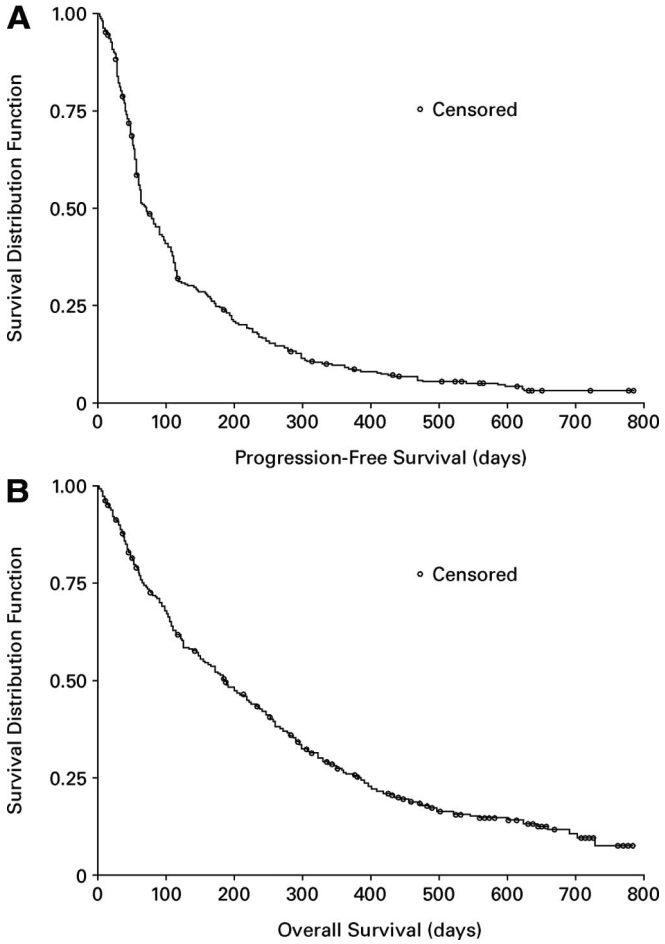

FIGURE 1. Progression-free (*A*) and overall survival (*B*) during treatment with erlotinib.

comes (as defined in the methods section; MW score = 0.66; $p = 0.001$, $n = 161$).

Sixty-one patients with data for both EGFR IHC and *EGFR* FISH also had response data. Seven had responses (2 CR, 5 PR), and all 7 were both IHC- and FISH-positive. Of the 54 patients with SD/PD, 31 were both IHC- and FISH-positive, while 23 tested positive for one marker or neither ($p = 0.04$, Fisher's exact test). There was a significant relationship between IHC/FISH 'double-positive' status and response-based clinical outcomes (MW score = 0.73, $p = 0.002$, $n = 61$).

EGFR mutations were detected in 6 of 92 patients (7%); L858R in four, and the E746-A750 deletion in two. One of these patients had CR, one PR, and 2 SD as best response (1 not evaluable; 1 no data). Patients with mutations had significantly longer PFS (hazard ratio [HR] = 0.31) and OS (HR = 0.33) than those with wild-type or indeterminate genes (Table 2, Figure 2) Five of the six tumors with *EGFR* mutations were adenocarcinomas; all were EGFR IHC-positive (four were *EGFR* FISH-positive). Four patients with mutations were never-smokers, and four were women. *EGFR* and *KRAS* mutations were mutually exclusive.

Seventeen patients (15%) had *KRAS* mutations; 15 at codon 12 (G12X), one at codon 13 (G13X) and one at codon 61 (Q61X). None of the patients had a response to erlotinib, but 6 had SD. The impact of *KRAS* mutation status on PFS and OS was of borderline statistical significance (Table 2 and Figure 2). Of the 17 patients with *KRAS* mutations, 16 were smokers, and 12 were men. None of the tumors were *EGFR* FISH-positive, 13 were EGFR IHC-positive and 15 were nonsquamous tumors.

Patients testing positive for pMAPK IHC had shorter PFS and OS than those with negative pMAPK IHC tests (Figure 3), but the difference in PFS was not significant and the difference in OS was of borderline significance (Table 2).

TABLE 2. Response and Survival According to Biomarker Status[a]

	Response						Survival				
	Positive		Negative		Odds Ratio			PFS		OS	
	n^b	%	n^b	%	(95% CI)	p^c	Positive/Negative	HR	p^d	HR	p^d
EGFR IHC (≥10%)	18/191	9.4	2/42	4.8	2.08 (0.46–9.34)	0.542	231/57	0.79 (0.59–1.06)	0.120	0.84 (0.61–1.14)	0.254
EGFR IHC (any)	19/208	9.1	1/25	4.0	2.41 (0.31–18.83)	0.705	252/36	0.73 (0.51–1.04)	0.081	0.73 (0.51–1.06)	0.097
EGFR FISH	7/41	17.1	7/120	5.8	3.32 (1.09–10.14)	0.048	49/157	0.58 (0.42–0.82)	0.002	0.63 (0.43–0.91)	0.012
EGFR mutation	2/4	50.0	2/68	2.9	33.0 (2.96–370.4)[e]	0.014	6/85[f]	0.31 (0.13–0.78)[e]	0.009[e]	0.33 (0.12–0.91)[e]	0.025[e]
KRAS mutation	0/11	0	7/78	9.0	na	0.590	17/96[f]	1.56 (0.92–2.65)[e]	0.094[e]	1.64 (0.97–2.80)[e]	0.064[e]
pMAPK IHC[g]	2/33	6.1	15/120	12.5	0.45 (0.10–2.08)	0.368	47/146	1.26 (0.90–1.77)	0.183	1.39 (0.98–1.99)	0.067
pAKT IHC[g]	9/42	21.4	5/110	4.5	5.73 (1.79–18.28)	0.003	53/137	0.80 (0.57–1.11)	0.184	0.98 (0.70–1.37)	0.896

[a] For patients with both biomarker data and response/survival data.
[b] Responders (CR + PR)/total.
[c] Fisher's exact test.
[d] Log-rank test.
[e] Excludes patients with indeterminate mutation status.
[f] Mutation/wild-type (101 indeterminate results for *EGFR*; 79 indeterminate results for *KRAS*).
[g] H-Score ≥200.

PFS, progression-free survival; OS, overall survival; CI, confidence interval; HR, hazard ratio; EGFR, epidermal growth factor receptor; IHC, immunohistochemistry; FISH, fluorescence in situ hybridization; na, not applicable; pMAPK, phosphorylated mitogen-activated protein kinase; pAKT, phosphorylated AKT.

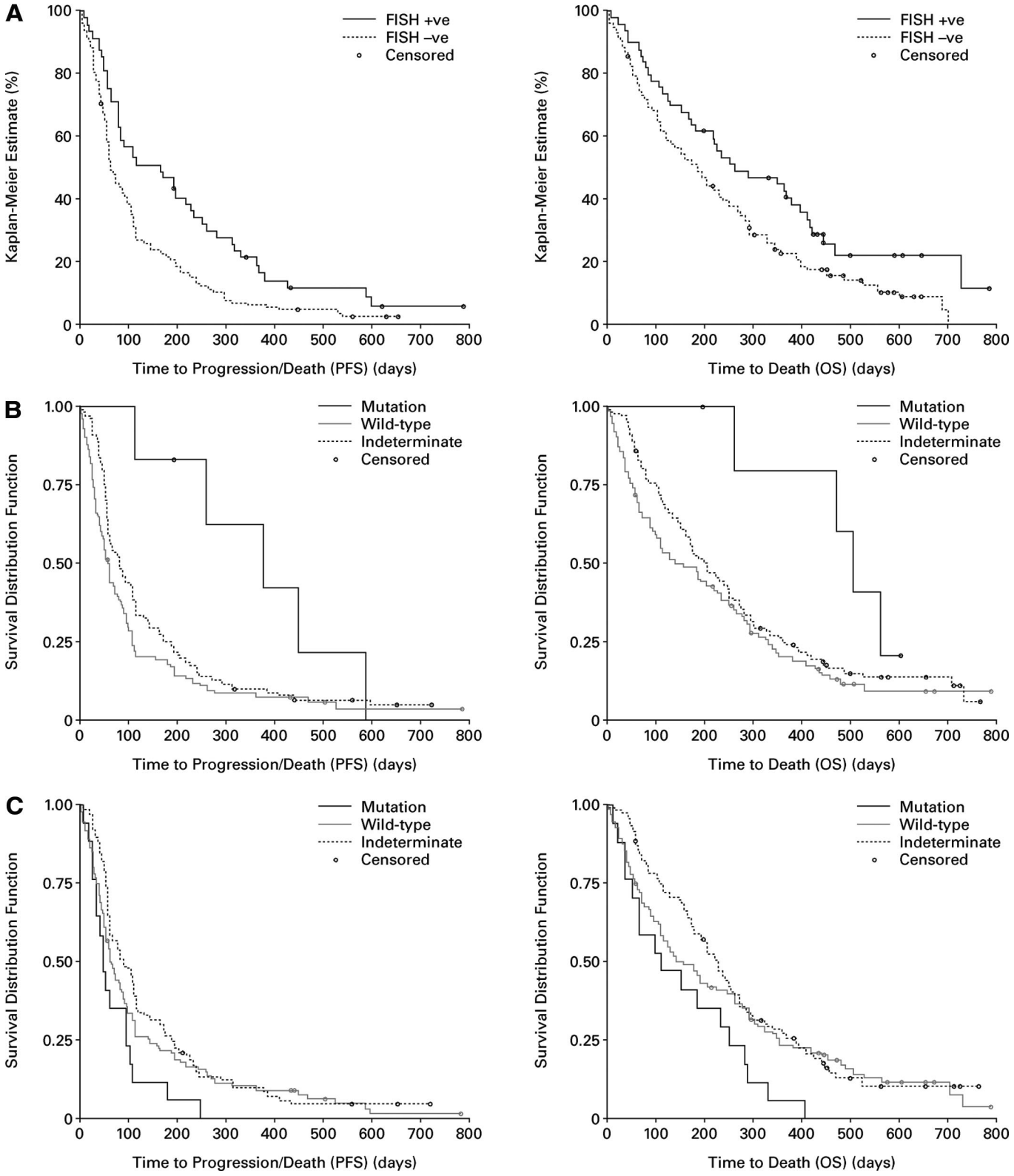

FIGURE 2. Survival outcomes according to tumor marker status. Epidermal growth factor receptor (EGFR), fluorescence in situ hybridization (FISH) (*A*), EGFR mutation analysis (*B*) and KRAS mutation analysis (*C*).

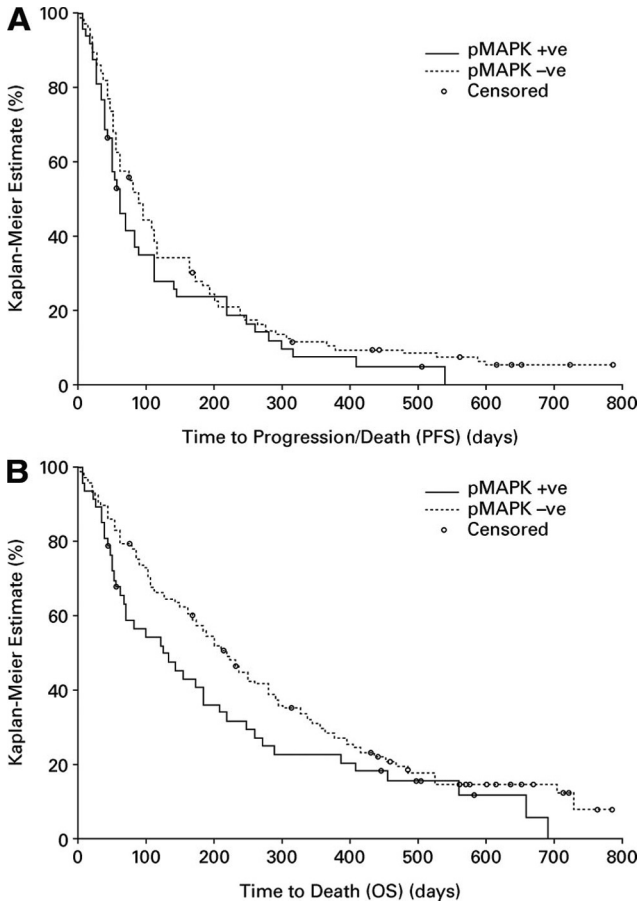

FIGURE 3. Progression-free survival (PFS) (*A*) and overall survival (OS) (*B*) according to phosphorylated mitogen-activated protein kinase (pMAPK) status (immunohistochemistry).

Median OS was 4.2 and 7.2 months in pMAPK IHC-positive and IHC-negative patients, respectively. pAKT IHC status had no detectable effect on PFS or OS (Table 2).

Relationship Between Smoking Status and Clinical Outcome

Never-smokers were more likely than former/current smokers to benefit; clinical outcome and smoking status was significantly correlated (MW score = 0.34, $p < 0.0001$). There were more women among the never-smokers (82%) than the smokers (28%), and this may have contributed to the difference in clinical outcome. A separate analysis found no significant association between gender and clinical outcome (male: MW score = 0.44, $p = 0.076$, $n = 311$). Also, 92% of patients with squamous-cell carcinoma were smokers, versus 66% of those with adenocarcinoma (Fisher's exact test: $p < 0.001$; Phi = 0.29; $n = 340$).

Significant differences in pAKT protein expression were observed between former/current and never-smokers. Forty-four percent of never-smokers were pAKT IHC-positive, versus 23% of smokers (Fisher's exact test; $p < 0.01$).

Relationship Between Skin Toxicity and Clinical Outcome

Of 392 patients with data available, 267 (68%) experienced rash, with the majority of these cases (218 patients; 82%) being grade 1 or 2. Both PFS and OS were significantly longer in patients who obtained grade ≥2 rash, compared with those who had grade 1 or no rash: median PFS 19.5 weeks versus 12.4 weeks, respectively (HR = 0.70, log-rank $p = 0.007$); median OS 9.7 weeks versus 5.9 weeks, respectively (HR = 0.68, log-rank $p = 0.005$).

Relationships Between Putative Biomarkers

The percentage of cells with EGFR IHC membrane staining increased with increasing Cappuzzo EGFR FISH stratum ($\rho = 0.25$, $p < 0.001$, $n = 208$, Spearman's rank correlation). This was also apparent when staining intensity was quantified using H-scores. Ninety-two percent (45 of 49) of EGFR FISH-positive tumors were EGFR IHC-positive ('any staining' cutoff; $p < 0.05$, Fisher's exact test), while 90% (36 of 40) of EGFR IHC-negative tumors were EGFR FISH-negative ($p < 0.05$, Fisher's exact test). EGFR FISH and IHC status were concordant in 81 of 208 patients (39%).

Thirty-two percent (48 of 151) of EGFR IHC-positive tumors were pAKT-positive and 25% were pMAPK-positive. No relationships were identified between pMAPK status and other markers (data not shown).

Relationships Between Tumor Histology and Biomarker Status

EGFR IHC-positive status was more common in squamous-cell carcinomas (89%; 84 of 94) than adenocarcinomas (78%; 125 of 161; $p < 0.05$, Fisher's exact test). The percentage of membranous staining was also greater in squamous cell carcinomas (MW score = 0.67; $p < 0.0001$; $n = 255$). Both adenocarcinomas (55%) and squamous-cell carcinomas (67%) were mainly EGFR IHC-positive and *EGFR* FISH-negative (Figure 4), but 21% of adenocarcinomas were EGFR IHC-negative and EGFR *FISH*-negative, versus 11% of squamous-cell carcinomas.

DISCUSSION

Findings from the BR.21 study suggested that putative biomarkers for outcomes with erlotinib were worthy of further investigation.[9–11] In this trial, we examined the relationships between certain previously reported candidate markers and clinical outcomes in patients receiving open-label treatment with erlotinib. We also examined two other components of EGFR-linked signaling, pAKT and pMAPK, as potential markers for outcomes with erlotinib in patients with NSCLC.

The outcomes with erlotinib in this study are generally consistent with those from the phase III erlotinib study.[6] The response rates were similar (7.9 and 8.9%, respectively), but here the disease control rate was somewhat higher (66% versus 45%). PFS and OS (2.3 and 6.1 months) were very similar to those in the double-blind setting (2.2 and 6.7 months, respectively).

As previously reported,[6] the response rate to erlotinib favored patients with *EGFR* mutations (but based on a small

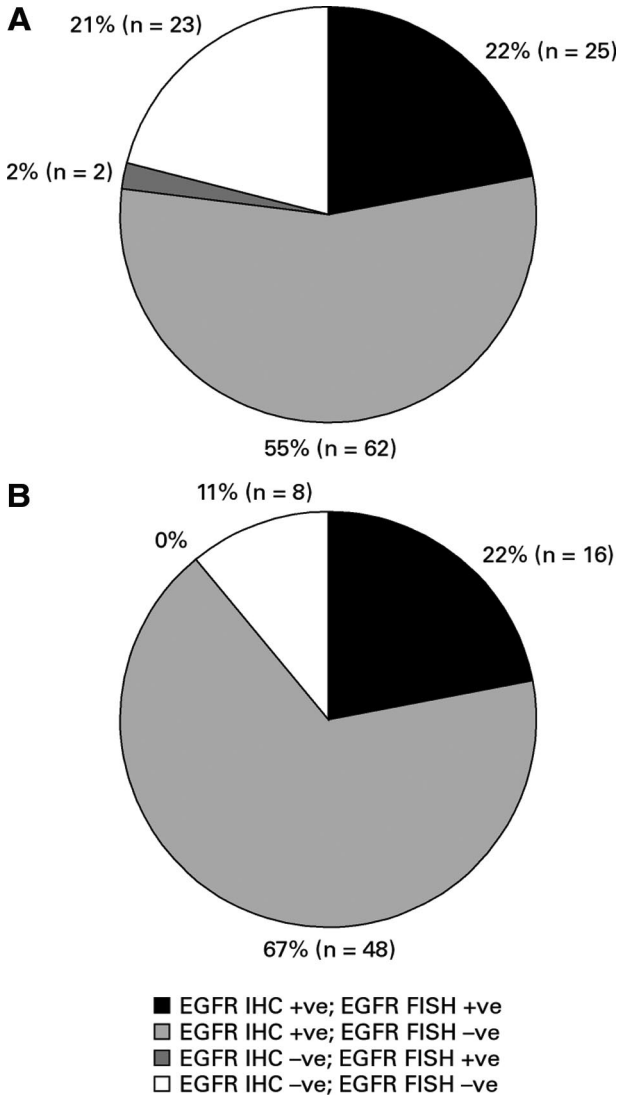

FIGURE 4. Epidermal growth factor receptor (EGFR), immunohistochemistry (IHC) and fluorescence in situ hybridization (FISH) status in adenocarcinomas (*A*) and squamous cell carcinomas (*B*).

number of patients) and, less markedly, in those with high *EGFR* gene copy number. According to a meta-analysis based on 1335 patients, the response rate to TKIs in patients with *EGFR* mutations is approximately 70%, versus 10% in those with wild-type EGFR,[15] but the proportion with clinical benefit is generally greater than the proportion with *EGFR* gene mutations.[6,16,17] In the current trial, 66% of patients had a clinical benefit, but only 7% had *EGFR* mutations.

Although patients with *EGFR* mutations had longer PFS and OS than those with wild-type *EGFR* or indeterminate status, there is currently no general agreement about the predictive value of *EGFR* mutations for survival with erlotinib. Patients with *EGFR* mutations may have prolonged survival, regardless of the treatment received.[18] Given the uncontrolled nature of the present study, and the small numbers of patients possessing an EGFR mutation, we are unable to determine whether the survival benefit in patients with *EGFR* mutations was treatment-related. In BR.21, *EGFR* mutation status was neither prognostic nor predictive.[11] To provide more definitive answers, it will be necessary to conduct large randomized controlled studies with prospective assessment of *EGFR* mutation status. Another approach currently being investigated by the Spanish Lung Cancer Group is the use of erlotinib as a first-line treatment for patients with *EGFR* mutations.

Our analysis indicates that patients with EGFR high polysomy or gene amplification had a significantly higher response rate to erlotinib, and longer OS and PFS. Shepherd et al.[11] reported that EGFR FISH-positive status was both prognostic for poorer survival ($p = 0.005$) and predictive for survival benefit with erlotinib ($p = 0.009$). In a randomized, open-label trial (IRESSA non-small cell lung cancer trial evaluating response and survival against Taxofere [INTEREST]) there was no notable difference in survival between gefitinib and docetaxel in any biomarker-defined subgroup, including patients classified by EGFR FISH status.[19] However, in INTEREST, the control group received docetaxel, but in BR.21 the control group received placebo, so the two studies are not directly comparable. In a randomized placebo-controlled trial of relapsed NSCLC, the effect of gefitinib on survival in EGFR FISH-positive patients was of borderline significance (HR = 0.61, $p = 0.067$), but no notable effect was observed in FISH-negative patients (HR = 1.16, $p = 0.417$).[20]

In this study, no patients with *KRAS* mutations had an objective tumor response. This was not surprising, as the presence of *KRAS* mutations seems to be a negative prognostic factor in NSCLC.[21,22] The hazard ratios for the effect of *KRAS* status on PFS and OS were high, but were not statistically significant (due to the limited number of samples). Among the 6 patients with *KRAS* mutations who had SD as best response, 4 had a grade 2 rash and PFS was between 3 and 8 months, approximately. In BR.21, the response rate was 5% in patients with *KRAS* mutations and 10% in those with the wild-type gene. The HRs for survival with erlotinib versus placebo were 1.67 and 0.69 respectively, but the difference was not significant ($p_{\text{interaction}} = 0.09$).[11] Further studies are needed to give a more definitive answer to the question of whether patients with *KRAS* mutations can derive survival benefit from erlotinib.

pMAPK-positive status may be a negative prognostic factor in NSCLC,[23,24] but, to our knowledge, this is the first study to examine the effect of pMAPK-IHC and pAKT status on outcomes with erlotinib in NSCLC. Patients with pMAPK IHC-positive tumors had shorter PFS and OS than those with pMAPK IHC-negative tumors, but the differences were not marked. The response rate on erlotinib favored patients with pAKT IHC-positive versus IHC-negative tumors, but this was not reflected by differences in PFS or OS.

Current data do not support selection of patients for treatment with erlotinib on the basis of tumor molecular characteristics.[7] Emerging data from ongoing studies, particularly the Sequential Tarceva in unresectable NSCLC (SATURN) trial, should provide further clarification. In BR.21,

most types of patients obtained a therapeutic benefit with erlotinib, irrespective of clinical or tumor molecular characteristics.[6,11] These findings were confirmed in the TRUST study, in which the clinical benefit from erlotinib was observed in a broad range of patient subgroups based on clinical characteristics.[25,26] Whether patients who are likely to benefit most from EGFR TKI therapy can be reliably identified is open to question. The aforementioned SATURN trial, a large, randomized, controlled study examining candidate biomarkers in a prospective manner, in patients receiving maintenance erlotinib following first-line chemotherapy, may help to provide a definitive answer.

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