ORIGINAL ARTICLE

Screening for Epidermal Growth Factor Receptor Mutations in Lung Cancer

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ABSTRACT

BACKGROUND

Activating mutations in the epidermal growth factor receptor gene (*EGFR*) confer hypersensitivity to the tyrosine kinase inhibitors gefitinib and erlotinib in patients with advanced non–small-cell lung cancer. We evaluated the feasibility of large-scale screening for *EGFR* mutations in such patients and analyzed the association between the mutations and the outcome of erlotinib treatment.

METHODS

From April 2005 through November 2008, lung cancers from 2105 patients in 129 institutions in Spain were screened for *EGFR* mutations. The analysis was performed in a central laboratory. Patients with tumors carrying *EGFR* mutations were eligible for erlotinib treatment.

RESULTS

EGFR mutations were found in 350 of 2105 patients (16.6%). Mutations were more frequent in women (69.7%), in patients who had never smoked (66.6%), and in those with adenocarcinomas (80.9%) ($P<0.001$ for all comparisons). The mutations were deletions in exon 19 (62.2%) and L858R (37.8%). Median progression-free survival and overall survival for 217 patients who received erlotinib were 14 months and 27 months, respectively. The adjusted hazard ratios for the duration of progression-free survival were 2.94 for men ($P<0.001$); 1.92 for the presence of the L858R mutation, as compared with a deletion in exon 19 ($P=0.02$); and 1.68 for the presence of the L858R mutation in paired serum DNA, as compared with the absence of the mutation ($P=0.02$). The most common adverse events were mild rashes and diarrhea; grade 3 cutaneous toxic effects were recorded in 16 patients (7.4%) and grade 3 diarrhea in 8 patients (3.7%).

CONCLUSIONS

Large-scale screening of patients with lung cancer for *EGFR* mutations is feasible and can have a role in decisions about treatment.

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Molecular-profiling studies indicate that activating mutations in the epidermal growth factor receptor (*EGFR*), *PI3K*, *BRAF*, and *K-ras* genes are generally nonoverlapping and identifiable in approximately 40% of non–small-cell lung cancers. These mutations, plus others that contribute to tumor progression ("driver" mutations), can be found in almost half of all non–small-cell lung cancers.[1,2]

The two proto-oncogenes that are most commonly mutated in pulmonary adenocarcinomas are *K-ras* and *EGFR*. Nearly 90% of lung-cancer–specific *EGFR* mutations comprise a leucine-to-arginine substitution at position 858 (L858R) and deletion mutants in exon 19 that affect the conserved sequence LREA (delE746-A750).[3-7] These mutations cause constitutive activation of the tyrosine kinase of the EGFR by destabilizing its autoinhibited conformation, which is normally maintained in the absence of ligand stimulation.[8] The activating mutations confer hypersensitivity to the tyrosine kinase inhibitors gefitinib and erlotinib.[3-5] In transgenic mouse models, *EGFR* mutations induced adenocarcinomas that responded to suppression of the EGFR driving signal and to EGFR tyrosine kinase inhibitors.[9,10] Furthermore, lung-cancer–specific *EGFR* mutants can transform fibroblasts and Ba/F3 cells.[11,12] A kinetic analysis of these mutations showed that exon 19 deletions are more sensitive to erlotinib inhibition than the L858R mutation,[13] a finding that has been confirmed in retrospective clinical studies.[14-16]

In retrospective studies, we[17,18] and others[14,19,20] found that *EGFR* mutations were an independent predictor of response, progression-free survival, and overall survival in patients with non–small-cell lung cancer who were treated with gefitinib; most of the patients in these studies had undergone previous chemotherapy. Consistent with previous findings,[6,7] *EGFR* mutations were more frequent in women, patients with adenocarcinomas, those who had never smoked, and Asians, all of whom also had the best response to gefitinib.[14,17,19,20] Two small, prospective, multicenter studies customized gefitinib as first-line therapy or after up to two previous chemotherapy regimens in patients with non–small-cell lung cancer with *EGFR* mutations.[21,22] Response rates were as high as 75%, and 1-year survival was as high as 79%.[21,22] At least five additional prospective, single-institution studies in Japan have reported similar outcomes.[23] However, all these studies had a relatively short observation period and included small numbers of selected patients. Moreover, most of the studies were carried out in Japan, whereas the incidence of *EGFR* mutations in Europe has not been defined.

We now report a prospective study of screening for *EGFR* mutations in patients with advanced non–small-cell lung cancer, conducted by the Spanish Lung Cancer Group. We registered patients from 129 centers from all regions of Spain and used a central laboratory and database. Patients with *EGFR* mutations were considered for customized erlotinib treatment, and we evaluated the association between *EGFR* mutations and outcome.

METHODS

PATIENTS

We prospectively screened 2105 patients with non–small-cell lung cancer for *EGFR* mutations. Patients with mutations were then considered for erlotinib treatment at a dose of 150 mg daily until disease progression or the advent of intolerable adverse effects. The registration of patients in the database, pathological review, and *EGFR* mutation assessment were performed centrally at the Catalan Institute of Oncology. Eligibility criteria were the diagnosis of stage IIIB disease with pleural effusion or stage IV non–small-cell lung cancer. The smoking history of the patients was obtained at baseline, and patients were categorized as those who had never smoked (<100 lifetime cigarettes), former smokers (≥1 year since cessation), or current smokers (still smoking, or <1 year since cessation).

All patients provided written informed consent. Approval was obtained from the institutional review board and the ethics committee at each hospital. Details on inclusion and exclusion criteria and on treatment and evaluation are provided in the Supplementary Appendix, available with the full text of this article at NEJM.org.

ASSESSMENT OF *EGFR* MUTATIONS

A total of 2105 samples of tumor tissue from patients with non–small-cell lung cancer were analyzed: 2060 paraffin-embedded tissues and 45 fresh specimens. All specimens were obtained from the original biopsy, before any treatment. Genomic DNA was derived from tumor tissue after laser capture microdissection (Palm Microlaser Technologies). Baseline blood samples were available from 164 patients. DNA from serum, plasma, or

both was isolated with the use of the QIAamp DNA Blood Mini Kit (Qiagen), starting from 0.4 ml of material.

For tissue samples, deletions in exon 19 (del 19) were determined by length analysis after polymerase-chain-reaction (PCR) amplification with the use of a FAM-labeled primer in an ABI Prism 3130 DNA Analyzer (Applied Biosystems). Exon 21 point mutations in codon 858 were detected with a 5′ nuclease PCR assay (TaqMan assay) using FAM and VIC MGB-labeled probes for the wild-type and the mutant sequence, respectively. All mutants were confirmed by DNA sequencing.[17,24] For blood samples, both reactions were performed in the presence of a protein nucleic acid (PNA) clamp, designed to inhibit the amplification of the wild-type allele. For L858R in exon 21, a PNA clamp was also added to the 5′ nuclease PCR reaction (TaqMan assay). (For additional details, see the Supplementary Appendix.)

STUDY OVERSIGHT

None of the funding agencies were involved in the study's design or conduct, data management or analysis, manuscript preparation or review, or the decision to submit the manuscript for publication. The erlotinib that was used in the study was purchased from the manufacturer, which had no role in the study.

STATISTICAL ANALYSIS

Approximately 18,800 new cases of lung cancer are diagnosed per year in Spain.[25] On the basis of our preliminary study (unpublished data), we expected approximately 15% of these patients to carry *EGFR* mutations. With an estimated error rate of 5%, we calculated that 2105 patients would need to be enrolled during a 3-year period for a power of 80%. We randomly chose 100 of the 879 public hospitals in Spain to contact with a request for samples for inclusion in the database. However, because of extensive media coverage of this project, other centers requested permission to send samples for inclusion in the database, and these samples were also included. All centers sent samples from patients who had received a diagnosis of lung cancer in a nonconsecutive manner, and there was no stratification according to sex, performance status, smoking history, or previous treatment. A database and a case-record form were designed and sent to all participating hospitals.

Progression-free survival was defined as survival without disease progression or death and was calculated from the start of erlotinib therapy until the first observation of disease progression. Survival was calculated from the start of erlotinib therapy until death or the last follow-up visit. The associations between *EGFR* status, clinical characteristics, and tumor response to erlotinib were analyzed with the use of the chi-square test or Fisher's exact test. The normality of quantitative variables was analyzed with the use of the Kolmogorov–Smirnov test and compared by Student's t-test analysis of variance or Mann–Whitney and Kruskal–Wallis tests. Confidence intervals were calculated with the use of binomial distribution. Progression-free survival and survival curves were constructed by the Kaplan–Meier method and compared with the use of the log-rank test and the Tarone–Ware test.

In addition to the principal analyses, we performed five post hoc analyses of patients' characteristics and response according to sex, smoking history, age, Eastern Cooperative Oncology Group (ECOG) performance status, and treatment. The Bonferroni method was used in multiple comparisons.

All statistical calculations were performed with the use of SPSS software (version 17.0) and S-Plus (version 6.1). Two-sided P values of less than 0.05 were considered to indicate statistical significance.

RESULTS

PATIENTS

From April 2005 through November 2008, a total of 2105 patients with non–small-cell lung cancer from 129 institutions were prospectively screened for *EGFR* mutations. The median time required for such analysis was 7 days (range, 5 to 9) from the time the sample arrived at the laboratory until the results were reported to the investigators. Mutations in the *EGFR* gene were detected in 350 of 2105 patients (16.6%). Mutations were found more frequently in women (69.7%), in patients who had never smoked (66.6%), and in those with adenocarcinomas (80.9%) (P<0.001 for all comparisons) (Table 1). Although no special call for an enriched population was made, the participating centers included more samples from women and patients who had never smoked, since physicians were aware that *EGFR* mutations are more frequent in these subgroups. We considered 296 patients with tumors carrying *EGFR* mutations for treatment with erlotinib; of these patients, 79 did not receive erlotinib for a variety of reasons (Fig. 1). Of the 217

Table 1. Frequency of *EGFR* Mutations.*

Variable	All Patients (N=2105)	Patients with *EGFR* Mutations (N=350)	Frequency of Mutations	
			All 2105 Patients	350 Patients with Mutations
	number of patients		*percent (95% CI)*	
Sex				
Female	814	244	30.0 (26.9–33.2)	69.7 (64.7–74.3)
Male	1287	106	8.2 (6.8–9.9)	30.3 (25.7–35.3)
Missing data	4	0		
Age				
<56.7 yr	638	89	13.9 (11.5–16.9)	27.1 (24.9–29.2)
56.7–69.1 yr	638	99	15.5 (12.9–18.6)	30.1 (27.8–32.4)
>69.1 yr	632	141	22.1 (19.1–25.6)	42.8 (40.2–45.5)
Missing data	197	21		
Smoking history				
Former smoker	958	91	9.5 (7.8–11.6)	26.2 (24.2–28.2)
Current smoker	424	25	5.8 (4.0–8.6)	7.2 (6.5–7.9)
Never smoked	612	231	37.7 (34.0–41.7)	66.6 (64.2–68.9)
Missing data	111	3		
Tumor type				
Adenocarcinoma	1634	283	17.3 (15.5–19.3)	80.9 (76.4–84.7)
Bronchioloalveolar adenocarcinoma	147	34	23.1 (17.0–30.7)	9.7 (7.0–13.3)
Large-cell carcinoma	287	33	11.5 (8.3–15.8)	9.4 (6.8–13.0)
Missing data	37	0		

* CI denotes confidence interval.

patients who received erlotinib, 197 could be evaluated for a response. *EGFR* mutations were also assessed in paired serum samples from the 164 patients for whom baseline blood samples were available (Table 2 and Fig. 1).

Table 2 shows characteristics of the 217 patients who received erlotinib. The median age was 67 years; most of the patients were white women who had never smoked and had an adenocarcinoma, with an ECOG performance status of 1. Of these patients, 113 received erlotinib as first-line therapy, and 104 received the drug as second- or third-line therapy. *EGFR* del 19 mutations were detected in 135 tumors, and the L858R mutation in 82 tumors. Of the 164 patients in whom *EGFR* mutations were assessed in serum, 97 carried mutations: del 19 in 64 patients and L858R in 33 patients. (For additional details on patients with an *EGFR* mutation, see the Supplementary Appendix.)

RESPONSE

Of the 197 patients who could be evaluated, 24 had a complete response, and 115 had a partial response; 38 had stable disease, and 20 had progressive disease (Table 2, and Tables 1 through 5 in the Supplementary Appendix). A better response was associated with the del 19 mutation than with the L858R mutation (odds ratio, 3.08; 95% confidence interval [CI], 1.63 to 5.81; P=0.001) and an age between 61 and 70 years (odds ratio, 2.55; 95% CI, 1.32 to 4.96; P=0.006).

PROGRESSION-FREE AND OVERALL SURVIVAL

Median follow-up was 14 months (range, 1 to 42). Median progression-free survival was 14.0 months (95% CI, 11.3 to 16.7) (Fig. 2A). The duration of response was similar for patients receiving first-line therapy (14.0 months; 95% CI, 9.7 to 18.3) and second-line therapy (13.0 months; 95% CI, 9.7 to

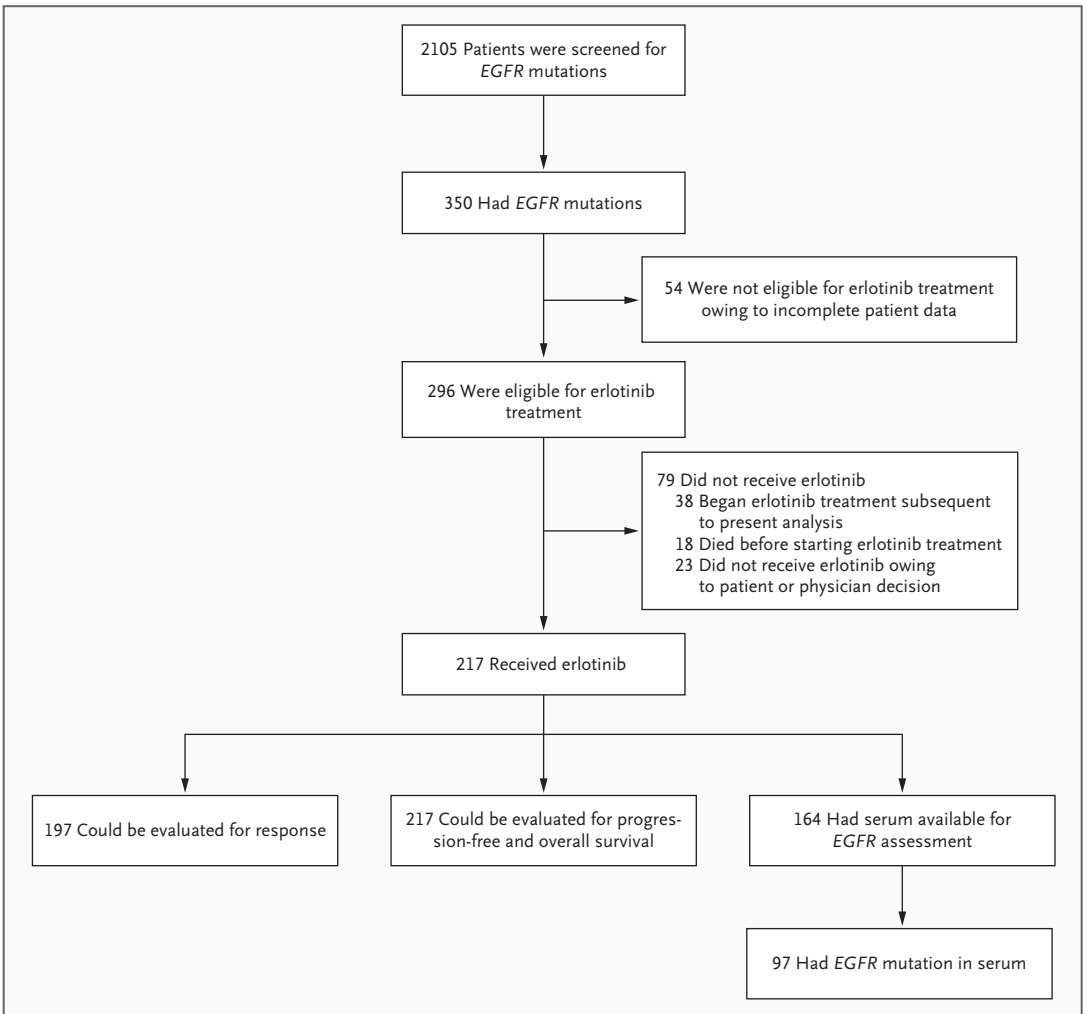

Figure 1. Enrollment and Outcomes.

16.3; P=0.62) (Fig. 2B). Median overall survival was 27.0 months (95% CI, 22.7 to 31.3) (Fig. 2C). Median overall survival for patients receiving first-line therapy was 28.0 months (95% CI, 22.7 to 33), and for those receiving second-line therapy, it was 27.0 months (95% CI, 19.9 to 34.1; P=0.67) (Fig. 2D).

Median progression-free survival was 16.0 months (95% CI, 12.7 to 19.2) in women and 9.0 months (95% CI, 6.1 to 11.9) in men (P=0.003). Median overall survival was 29.0 months (95% CI, 24.9 to 33.1) in women and 18.0 months (95% CI, 14.5 to 21.5) in men (P=0.05) (Fig. 1A and 1B in the Supplementary Appendix). There were no significant differences in progression-free survival according to performance status, age, first-line therapy versus second-line or third-line therapy, smoking history, or type of mutation (data not shown). (For details regarding differences observed in specific subgroups and for differences according to response, see the Supplementary Appendix.)

In a multivariate analysis (including sex, smoking status, performance status, first-line therapy vs. second-line or third-line therapy, del 19 vs. L858R, the presence or absence of brain or bone metastases, and the presence or absence of *EGFR* mutations in serum DNA), there was an association between poor progression-free survival and male sex (hazard ratio, 2.94; 95% CI, 1.72 to 5.03; P<0.001) and the presence of the L858R mutation (hazard ratio, 1.92; 95% CI, 1.19 to 3.10; P=0.02). In the multivariate analysis of overall survival, an ECOG performance status of 1, male

sex, the presence of the L858R mutation, and the diagnosis of bronchioloalveolar adenocarcinoma were associated with poor prognosis (Table 3).

THERAPY AFTER DISEASE PROGRESSION

A total of 55 patients received additional treatment after the discontinuation of erlotinib: 49% received cisplatin-based chemotherapy; 25.5% received single-agent chemotherapy; 14.5% received erlotinib plus vorinostat, fulvestrant, or bevacizumab; and 11% received neratinib (HKI-272). The objective response rate for first-line post-erlotinib treatment was 33%, including one complete and nine partial remissions. For 11 patients receiving a second-line post-erlotinib treatment, the response rate was 40%. Median survival for all 55 patients was 29.0 months (95% CI, 20.2 to 31.4); survival for the 159 patients who did not receive post-erlotinib treatment was 27.0 months (95% CI, 22.4 to 31.6; P=0.48).

ADVERSE EVENTS

The most common adverse events were skin rashes in 151 patients (69.6%) and diarrhea in 95 patients (43.8%); most events were grade 1 or 2 in severity. Grade 3 skin toxic effects were recorded in 16 patients (7.4%) and grade 3 diarrhea in 8 patients (3.7%). One 62-year-old man with del 19 had interstitial lung disease 1 month after the start of erlotinib, resulting in temporary interruption of treatment with the drug; he recovered with corticosteroid therapy and reinitiated erlotinib therapy at a lower dose. No patient was withdrawn from the study because of adverse events (Table 7 in the Supplementary Appendix).

DISCUSSION

We prospectively examined 2105 patients from Spain with adenocarcinoma of the lung and determined that 350 carried *EGFR* mutations (16.6%). Mutations were more frequent in women who had never smoked and in those with adenocarcinomas. In Europe, the only report of lung-cancer–specific *EGFR* mutations to date involved two sites in Italy, where *EGFR* mutations were found in 10% of 375 lung adenocarcinomas but in none of 31 large-cell carcinomas.[26] In our study, *EGFR* mutations were also found in 33 of 287 large-cell carcinomas.

The overall rate of complete or partial response to erlotinib was 70.6%, akin to that reported for gefitinib in retrospective[14,17-20] and prospective[21-23] studies. A higher probability of response was as-

Table 2. Characteristics and Treatment Responses of 217 Patients Receiving Erlotinib.*

Variable	Value
Age — yr	
Median	67
Range	22–88
Sex — no. (%)	
Male	59 (27.2)
Female	158 (72.8)
Race — no. (%)†	
Black	3 (1.4)
Asian	1 (0.5)
White	213 (98.2)
Smoking history — no. (%)	
Former smoker	56 (25.8)
Current smoker	13 (6.0)
Never smoked	148 (68.2)
ECOG performance status — no. (%)	
0	51 (23.5)
1	128 (59.0)
≥2	38 (17.5)
Tumor type — no. (%)	
Adenocarcinoma	176 (81.1)
Bronchioloalveolar adenocarcinoma	22 (10.1)
Large-cell carcinoma	19 (8.8)
Tumor stage — no. (%)	
IIIB	12 (5.5)
IV	205 (94.5)
Erlotinib therapy — no. (%)	
First line	113 (52.1)
Second or third line	104 (47.9)
EGFR mutation — no. (%)	
del 19	135 (62.2)
L858R	82 (37.8)
EGFR mutation in serum — no. (%)‡	
del 19	64 (39.0)
L858R	33 (20.1)
Not detected	67 (40.9)
Response — no. (%)§	
Complete response	24 (12.2)
Partial response	115 (58.4)
Complete or partial response	139 (70.6)
Stable disease	38 (19.3)
Progressive disease	20 (10.2)
Stable or progressive disease	58 (29.4)

* ECOG denotes Eastern Cooperative Oncology Group.
† Race was self-reported.
‡ The *EGFR* mutation was evaluated in the serum of 164 patients.
§ The response to erlotinib therapy was evaluated in 197 patients.

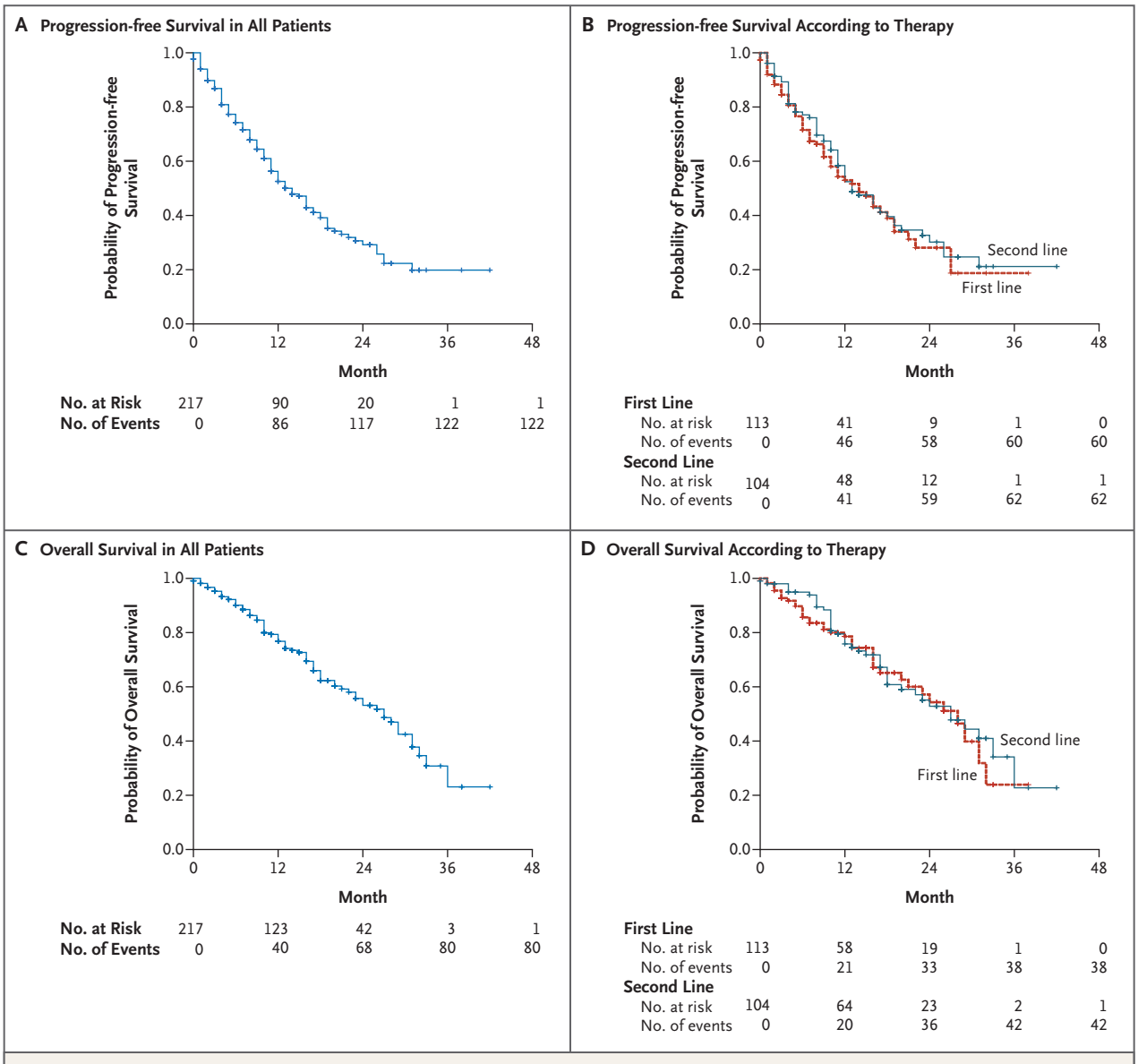

Figure 2. Kaplan–Meier Curves of Progression-free and Overall Survival.

Survival curves for 217 patients with *EGFR* mutations who received erlotinib therapy indicate the probability of progression-free survival (Panel A) and overall survival (Panel C) among all the patients and among those who received it as either first-line therapy or second-line therapy (Panels B and D). The cumulative number of events is listed for each time point.

sociated with del 19 (odds ratio, 3.08; 95% CI, 1.63 to 5.81; P=0.001) and an age between 61 and 70 years (odds ratio, 2.55; 95% CI, 1.32 to 4.96; P=0.006) but not with other factors. Overall, median progression-free survival for the 217 patients treated with erlotinib (as first-, second-, or third-line therapy) was 14 months, and median overall survival was 27 months, which is an improvement over findings in patients with lung cancer that have been reported previously. These results highlight the idea that *EGFR*-mutant lung cancer is a distinct class of non–small-cell lung cancer; in patients who do not have this mutation, chemotherapy normally yields a 30% response, a 5-month progression-free survival, and a 12-month median survival.[27] Outcomes in our study were not influ-

Table 3. Multivariate Analyses of Progression-free and Overall Survival.*

Variable	Progression-free Survival		Overall Survival	
	Hazard Ratio (95% CI)	P Value	Hazard Ratio (95% CI)	P Value
Sex				
Female	1.00		1.00	
Male	2.94 (1.72–5.03)	<0.001	3.48 (1.76–6.91)	<0.001
EGFR mutation				
del 19	1.00		1.00	
L858R	1.92 (1.19–3.10)	0.02	2.98 (1.48–6.04)	0.002
EGFR in serum				
Wild type	1.00		1.00	
Mutated	1.48 (0.93–2.36)	0.09	1.50 (0.82–2.74)	0.19
ECOG performance status				
0	1.00		1.00	
1	1.48 (0.85–2.58)	0.16	3.50 (1.42–8.66)	0.006
≥2	1.12 (0.52–2.45)	0.76	3.04 (0.95–9.75)	0.06
Age				
<60 yr	1.00		1.00	
60–70 yr	0.80 (0.43–1.47)	0.48	0.50 (0.21–1.19)	0.12
>70 yr	0.88 (0.47–1.67)	0.71	0.96 (0.41–2.26)	0.93
Smoking history				
Former smoker	0.72 (0.39–1.32)	0.29	0.70 (0.32–1.54)	0.38
Current smoker	1.65 (0.69–3.96)	0.26	1.37 (0.45–4.22)	0.58
Never smoked	1.00		1.00	
Tumor type				
Adenocarcinoma	1.00		1.00	
Bronchioloalveolar adenocarcinoma	1.86 (0.90–3.85)	0.09	2.82 (1.07–7.38)	0.03
Large-cell carcinoma	1.15 (0.56–2.37)	0.70	0.81 (0.29–2.22)	0.68
Tumor stage				
IIIB	1.00		1.00	
IV	0.81 (0.32–2.02)	0.65	0.74 (0.21–2.65)	0.74
Erlotinib therapy				
First line	1.00		1.00	
Second line	0.80 (0.50–1.28)	0.36	0.92 (0.48–1.73)	0.79
Third line	1.22 (0.51–2.87)	0.66	0.44 (0.14–1.44)	0.18
Brain metastases				
No	1.00		1.00	
Yes	1.07 (0.53–2.18)	0.84	2.28 (1.07–4.83)	0.03
Bone metastases				
No	1.00		1.00	
Yes	1.37 (0.84–2.24)	0.21	1.30 (0.68–2.51)	0.42

* ECOG denotes Eastern Cooperative Oncology Group.

enced by smoking status or previous chemotherapy, which is in line with the results of a small phase 2 trial of gefitinib.[21] (For details on planned genetic analyses, see the Supplementary Appendix.)

In conclusion, screening for *EGFR* mutations is warranted in women with lung cancer, in those who have never smoked, and in those with nonsquamous tumors. Large-scale screening of patients for *EGFR* mutations, with subsequent customization of erlotinib, is feasible and improves the outcome.

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No potential conflict of interest relevant to this article was reported.

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APPENDIX

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