

ORIGINAL ARTICLE

## Erlotinib in Lung Cancer — Molecular and Clinical Predictors of Outcome

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### ABSTRACT

#### BACKGROUND

A clinical trial that compared erlotinib with a placebo for non–small-cell lung cancer demonstrated a survival benefit for erlotinib. We used tumor-biopsy samples from participants in this trial to investigate whether responsiveness to erlotinib and its impact on survival were associated with expression by the tumor of epidermal growth factor receptor (EGFR) and *EGFR* gene amplification and mutations.

#### METHODS

EGFR expression was evaluated immunohistochemically in non–small-cell lung cancer specimens from 325 of 731 patients in the trial; 197 samples were analyzed for *EGFR* mutations; and 221 samples were analyzed for the number of *EGFR* genes.

#### RESULTS

In univariate analyses, survival was longer in the erlotinib group than in the placebo group when EGFR was expressed (hazard ratio for death, 0.68;  $P=0.02$ ) or there was a high number of copies of *EGFR* (hazard ratio, 0.44;  $P=0.008$ ). In multivariate analyses, adenocarcinoma ( $P=0.01$ ), never having smoked ( $P<0.001$ ), and expression of *EGFR* ( $P=0.03$ ) were associated with an objective response. In multivariate analysis, survival after treatment with erlotinib was not influenced by the status of EGFR expression, the number of *EGFR* copies, or *EGFR* mutation.

#### CONCLUSIONS

Among patients with non–small-cell lung cancer who receive erlotinib, the presence of an *EGFR* mutation may increase responsiveness to the agent, but it is not indicative of a survival benefit.

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**T**HE EPIDERMAL GROWTH FACTOR RECEPTOR family of genes (*EGFR*) encodes widely expressed transmembrane molecules that have been implicated in the development and progression of cancer.<sup>1-4</sup> After ligand binding, the transmembrane receptor forms homodimers or heterodimers, internalizes, and autophosphorylates tyrosine residues in its cytoplasmic domain, thereby triggering a cascade that leads to cellular proliferation, angiogenesis, metastasis, and inhibition of apoptosis.<sup>2</sup>

The *EGFR* gene is frequently expressed in solid tumors, and in some tumors, expression of the gene correlates with a poor clinical outcome.<sup>5</sup> Non-small-cell lung cancer frequently expresses *EGFR*,<sup>6-14</sup> and for this reason, it is of considerable interest for clinical trials of inhibitors of the tyrosine kinase of *EGFR*.<sup>4,15</sup> The kinase inhibitors erlotinib (Tarceva, OSI Pharmaceuticals) and gefitinib (Iressa, AstraZeneca) have been studied most extensively in clinical trials.<sup>15-18</sup>

Somatic mutations in the region of *EGFR* that encodes the tyrosine kinase domain of the receptor (exons 18 through 21) have been identified in lung cancer,<sup>19-21</sup> and many studies suggest that they can be used to predict responsiveness to gefitinib and erlotinib.<sup>19-30</sup> Such mutations occur more frequently in patients with adenocarcinoma, women, Asians, and patients who have never smoked. Whether *EGFR* mutations are more accurate predictors of responsiveness to inhibitors of *EGFR* than are these clinical factors has not been established. The effect of an *EGFR* mutation on prognosis and survival after treatment with an *EGFR* inhibitor is unclear, since studies to date have not included an untreated control group. The presence of mutations that affect extracellular domains of the receptor<sup>31</sup> does not predict outcome, and it is not known whether some of the newly identified mutations of the tyrosine kinase region are superior to others in predicting responsiveness. Stephens et al.<sup>32</sup> recently identified mutations in the kinase domain of the gene for the growth factor receptor HER2 in 4 percent of non-small-cell lung cancer tumors (10 percent of adenocarcinomas), but their clinical significance is unknown.

The National Cancer Institute of Canada Clinical Trials Group (NCIC CTG) BR.21 placebo-controlled study demonstrated a survival advantage for patients with non-small-cell lung cancer who received erlotinib after other treatments had failed.<sup>33</sup> Women, Asians, patients with adenocarcinoma,

and patients who had never smoked were more likely than other patients to have a response to erlotinib; however, those who had never smoked had a significant survival benefit from erlotinib. To clarify the role of *EGFR* in the outcome of non-small-cell lung cancer, we evaluated the expression of *EGFR* protein, the number of copies of *EGFR*, and mutation status of the gene in a subgroup of patients in the BR.21 study.

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## METHODS

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### CLINICAL STUDY

The BR.21 study was a phase 3 trial of erlotinib involving patients who had had progression after standard chemotherapy for non-small-cell lung cancer.<sup>33</sup> Patients were randomly assigned in a 2:1 ratio to receive 150 mg of erlotinib daily (OSI Pharmaceuticals) or placebo. The primary end point was overall survival. Response<sup>34</sup> was a secondary end point. Separate written consent was obtained for optional tissue banking and correlative studies. All studies were designed, executed, and analyzed by the NCIC CTG; the database was maintained by the NCIC CTG; and the manuscript was written by members of the NCIC CTG. OSI Pharmaceuticals reviewed the final manuscript.

### PATHOLOGY, ANALYSIS OF EXPRESSION OF *EGFR*, AND MOLECULAR ANALYSES

Interpretation of all *EGFR* analyses was blinded with respect to clinical response and demographic information. Paraffin blocks or 10 to 20 unstained slides were collected from diagnostic or resection specimens. The presence of adequate tumor tissue was verified by the study pathologist. The expression of *EGFR* protein was determined by means of immunohistochemistry with the use of Dako *EGFR* PharmDx kits (DakoCytomation). When more than 10 percent of tumor cells demonstrated membranous (partial or complete) staining of any intensity, the tumor was considered positive for *EGFR*.

The entire 5- $\mu$ m tissue section of specimens with cellularity of more than 50 percent was scraped from the slide for DNA isolation and mutational analyses. For specimens with lesser degrees of tumor cellularity or uneven distribution of tumor cells, enriched DNA was isolated from tumor cells that were microdissected from sections stained with toluidine blue (Fisher Canada) with the use of a dissecting microscope (model SZPT40, Olympus) at 40 $\times$  magnification. In some cases, we used laser

**Table 1. Baseline Demographic Characteristics of All Patients and the Patients Who Underwent Immunohistochemical (IHC) Analysis, Fluorescence in Situ Hybridization, and Mutational Analysis.\***

Characteristic	IHC Analysis (N=325)	FISH (N=125)	Mutational Analysis (N=177)	All Patients (N=731)
Hazard ratio for death (95% CI)†	0.76 (0.59–0.97)	0.63 (0.42–0.96)	0.71 (0.50–0.99)	0.70 (0.58–0.85)
	<i>number of patients (percent)</i>			
Age				
<60 yr	149 (46)	49 (39)	79 (45)	332 (45)
≥60 yr	176 (54)	76 (61)	98 (55)	399 (55)
Sex				
Female	115 (35)	44 (35)	66 (37)	256 (35)
Male	210 (65)	81 (65)	111 (63)	475 (65)
Performance status				
0 or 1	203 (62)	91 (73)	127 (72)	486 (66)
2 or 3	122 (38)	34 (27)	50 (28)	245 (34)
P value	0.04	—	—	—
Histopathological subtype				
Adenocarcinoma	164 (50)	67 (54)	95 (54)	365 (50)
Other	161 (50)	58 (46)	82 (46)	366 (50)
Race or ethnic group				
Asian	20 (6)	8 (6)	12 (7)	91 (12)
Other	305 (94)	117 (94)	165 (93)	640 (88)
P value	<0.001	0.03	0.01	—
Smoking history				
Current	49 (15)	15 (12)	20 (11)	110 (15)
Former	181 (56)	74 (59)	104 (59)	404 (55)
None	68 (21)	32 (26)	42 (24)	146 (20)
Unknown	27 (8)	4 (3)	11 (6)	71 (10)
P value	—	0.02	—	—
Prior cisplatin				
Yes	301 (93)	118 (94)	166 (94)	678 (93)
No	24 (7)	7 (6)	11 (6)	53 (7)
No. of prior regimens				
1	140 (43)	44 (35)	61 (34)	364 (50)
2 or 3	185 (57)	81 (65)	116 (66)	367 (50)
P value	0.001	<0.001	<0.001	—
Best response to prior chemotherapy				
Complete or partial response	133 (41)	59 (47)	76 (43)	292 (40)
Stable disease	137 (42)	52 (42)	80 (45)	287 (39)
Progression	55 (17)	14 (11)	21 (12)	152 (21)
P value	—	0.01	0.003	—
Time from diagnosis to randomization				
≤12 mo	134 (41)	52 (42)	65 (37)	339 (46)
>12 mo	191 (59)	73 (58)	112 (63)	392 (54)
P value	0.01	—	0.003	—
Decrease in body weight				
<5%	210 (65)	84 (67)	116 (66)	486 (67)
≥5%	99 (30)	34 (27)	47 (26)	213 (29)
Data missing	16 (5)	7 (6)	14 (8)	32 (4)
P value	—	—	0.03	—

\* P values reflect significance in a subgroup as compared with the entire study population. FISH and mutational analysis included only patients whose results could be evaluated. CI denotes confidence interval.

† The comparison group is the placebo group.

capture microdissection with a PixCell II System (Arcturus Bioscience). After proteinase K digestion, DNA was isolated according to the phenol–chloroform protocol. Exons 18 through 21 of the *EGFR* gene were sequentially amplified by two rounds of polymerase-chain-reaction (PCR) assays with the use of AmpliTaq Gold (Applied Biosystems) and external and internal primer sets designed by Paez et al.<sup>20</sup> Purified PCR products were sequenced in both directions with the use of the BigDye Terminator Cycle Sequencing Kit (version 3.1, Applied Biosystems) and an ABI Genetic Analyzer (model 3100, Applied Biosystems). Sequence data were analyzed by means of SeqScape software (version 2.1.1, Applied Biosystems), followed by manual review. Only sequence variations that were present in both directions in more than 15 percent of specimens were included in the analysis. When sufficient material was available, a second PCR assay was performed.

Fluorescence in situ hybridization (FISH) studies were performed with the use of dual-color DNA FISH probes containing the LSI *EGFR* (Vysis) probe specific for the *EGFR* locus (7p12) labeled with Spectrum Orange (Vysis) and the *CEP7* chromosome 7 centromere (7p11.1 through q11.1) probe labeled with Spectrum Green (Vysis). We analyzed 33 to 100 nonoverlapping tumor-cell nuclei to determine the number of red (*EGFR*) and green (*CEP7*) signals observed as well as the pattern of distribution of signals. We also determined the number of copies of *EGFR* and classified them according to the six FISH categories defined by Cappuzzo et al.<sup>22</sup> Samples with a high number of copies of *EGFR* (high degrees of polysomy or amplification) were considered to be FISH-positive.

#### STATISTICAL ANALYSIS

Exploratory analyses were performed to characterize the relationships between *EGFR* status and baseline clinical characteristics and outcomes with the use of the chi-square or Fisher's exact test. Cox regression models were used to correlate outcomes according to the time to an event, and logistic-regression models were used to correlate response to *EGFR* status and other baseline factors. All 731 randomized patients were included in survival analyses, and all 427 patients with measurable disease who were treated with erlotinib were included in analyses of the response. All reported P values are two-sided.

## RESULTS

### PATIENTS

Between August 2001 and January 2003, 731 patients were enrolled: 488 were assigned to receive erlotinib, and 243 to receive placebo. Biopsy tissue was available from 532 patients, but only 472 patients (313 in the erlotinib group and 159 in the placebo group) consented to tissue banking. A tissue sample adequate for at least one analysis was available from 328 patients (212 in the erlotinib group and 116 in the placebo group). The characteristics of samples that, after pathological review, contained sufficient tumor cells to attempt mutational and FISH analyses are described in Table 1 of the Supplementary Appendix (available with the full text of this article at [www.nejm.org](http://www.nejm.org)).

Table 1 shows the baseline characteristics of all patients and those who underwent *EGFR* testing. Although there were significant differences in some characteristics between patients who underwent various *EGFR* tests and the study population as a whole, the benefit of erlotinib, as compared with placebo, was similar in both the entire study group (hazard ratio for death, 0.70;  $P < 0.001$ ) (Fig. 1A) and the subgroup that underwent at least one *EGFR* analysis (hazard ratio, 0.76;  $P = 0.03$ ) (Fig. 1B).

### EXPRESSION OF *EGFR*, NUMBER OF COPIES OF *EGFR*, AND *EGFR* MUTATIONS

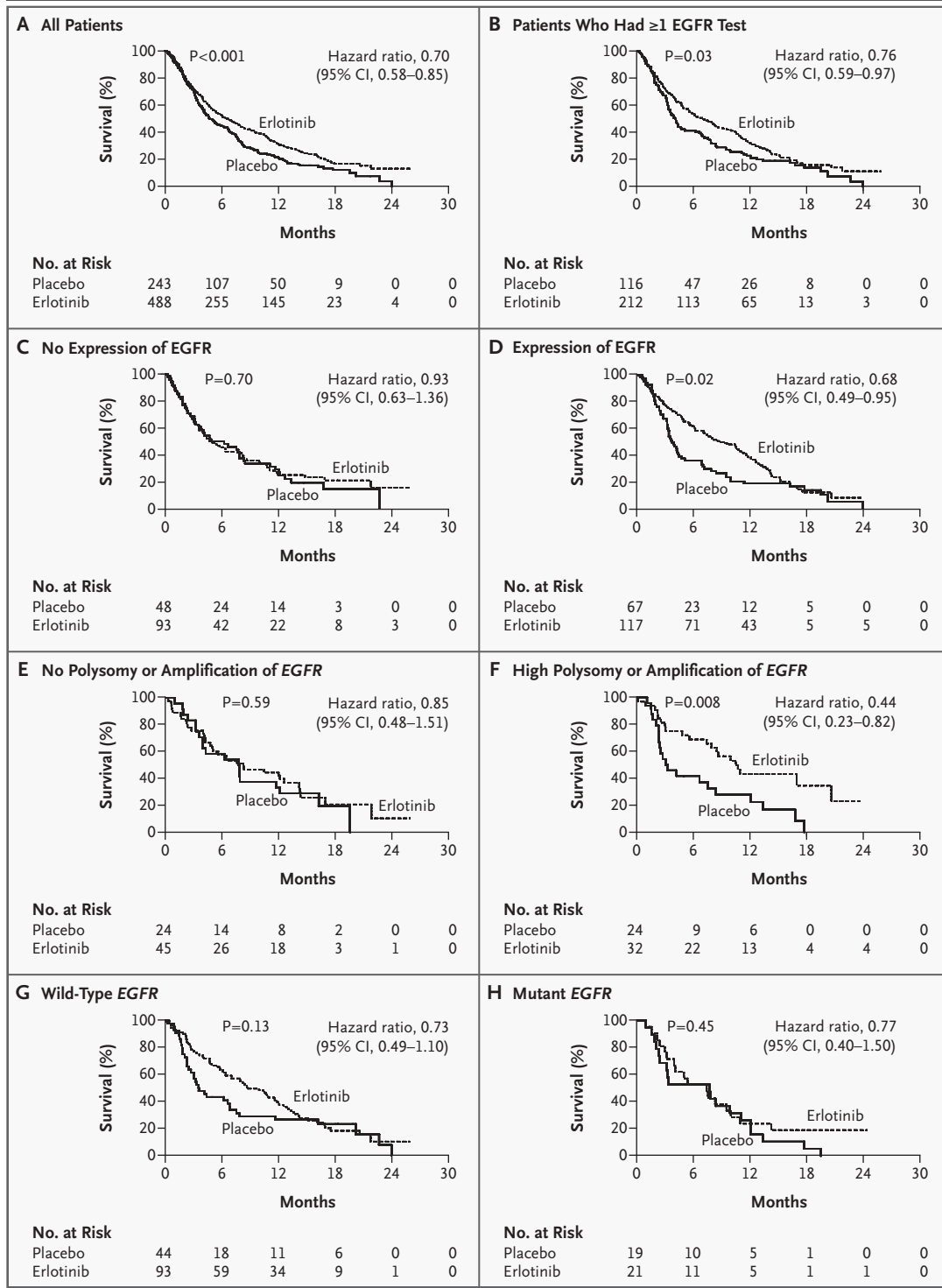
Among 325 tumors subjected to immunohistochemical analysis (Fig. 2 and Table 2), 184 (57 per-

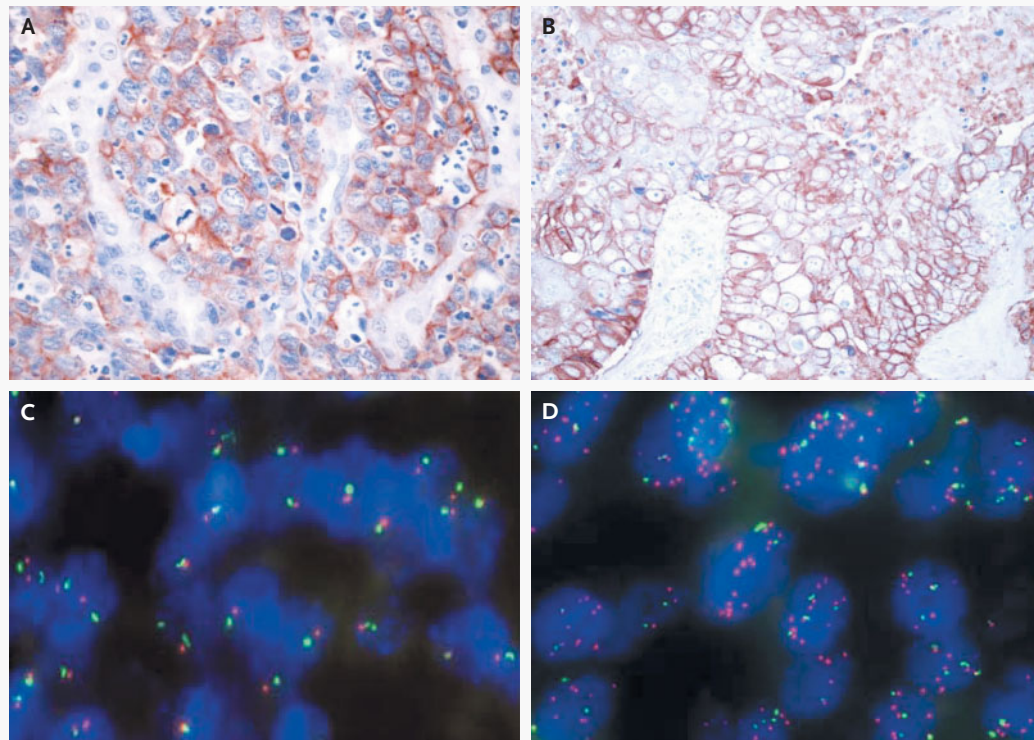
#### Figure 1 (facing page). Kaplan–Meier Estimates of Survival.

Panel A shows the results for all 731 study patients. Panel B shows the results for the 328 patients who had at least one *EGFR* analysis. Panel C shows the results for patients who did not have expression of *EGFR* on immunohistochemical analysis (less than 10 percent of tumor cells had membranous staining). Panel D shows the results for patients who had expression of *EGFR* on immunohistochemical analysis (10 percent or more of tumor cells had membranous staining). Panel E shows the results for patients who did not have *EGFR* amplification or high polysomy (four or more copies of *EGFR* in at least 40 percent of cells). Panel F shows the results for patients who had *EGFR* amplification or high polysomy. Panel G shows the results for patients who had wild-type *EGFR*. Panel H shows the results for patients who had *EGFR* mutations. P values were calculated with the use of a stratified log-rank test in Panel A and the log-rank test in Panels B through H.

cent) were EGFR-positive (50 percent of adenocarcinoma samples and 63 percent of samples of other types of tumors). FISH was attempted in 221 tumors and was successful in 125 (57 percent) (Fig. 2

and Table 2). Of these, 45 percent had high polysomy or amplification (48 percent of adenocarcinoma samples and 41 percent of samples of other types of tumors).





**Figure 2.** Expression of EGFR Protein on Immunohistochemical Analysis (Panels A and B) and Fluorescence in Situ Hybridization Analysis of the Number of Gene Copies (Panels C and D).

Incomplete (Panel A) and complete (Panel B) membranous immunohistochemical staining are scored as positive. FISH of diploid tumor cells shows one or two red (*EGFR*) and one or two green (*CEP7*) signals in most cells (Panel C), whereas cells with amplification show an excess of red signals (Panel D).

Mutational analyses were attempted in 197 samples, 110 of which yielded sufficient DNA to amplify and sequence exons 18 through 21 (59 required microdissection). Among these 110 samples, the analysis was unsuccessful in 3 samples and exons 19 and 21 were successfully analyzed in 107 samples, 24 of which (22 percent) contained one or more mutations. The 83 samples without mutations in exons 19 and 21 were classified as wild type, although some analyses of exons 18 (6 samples) and 20 (7 samples) had failed. The remaining 87 biopsy specimens of the original 197 contained small amounts of tissue (1 to 3 mm in diameter); microdissection was required for 45, since only 20 to 30 percent of cells were malignant. These 87 samples yielded DNA that was adequate for analysis of exons 19 and 21 alone. Analysis of 17 of the specimens was considered unsuccessful because one of these exons had no mutations and the other failed

to yield a definitive result. Analysis of the remaining 70 specimens revealed 17 mutations in 16 samples (23 percent). Thus, mutational analysis was successful in 177 of the 197 tumor specimens that were evaluated (90 percent).

In total, 45 mutations were found in 40 patients: 3 mutations in exon 18, 13 deletions and 8 mutations in exon 19, 5 mutations in exon 20, and 16 mutations in exon 21 (Fig. 3 and Table 2 in the Supplementary Appendix). Mutations were found in 28 percent of adenocarcinoma samples examined, 16 percent of tumor specimens of other histologic types ( $P=0.05$ ), 24 percent of specimens from women, 22 percent of specimens from men, 31 percent of specimens from patients who had never smoked, 21 percent of specimens from patients who were current or former smokers, 50 percent of specimens from Asian patients, and 21 percent of specimens from patients in other racial or ethnic

groups ( $P=0.03$ ). The presence of a mutation was not correlated with the expression of EGFR or the number of copies of EGFR.

#### RESPONSIVENESS TO ERLOTINIB

Univariate analysis of data from 427 patients who could be evaluated and who had received erlotinib (Table 3) showed that the following clinical features were significantly associated with responsiveness to erlotinib: female sex ( $P=0.007$ ), Asian origin ( $P=0.02$ ), never having smoked ( $P<0.001$ ), adenocarcinoma ( $P<0.001$ ), and polysomy or amplification of EGFR ( $P=0.03$ ). Mutational status had no significant association with responsiveness: 7 percent of those with wild-type EGFR had a response, as compared with 16 percent of those with an EGFR mutation ( $P=0.37$ ). Multiple logistic-regression analyses revealed that only never having smoked ( $P<0.001$ ), adenocarcinoma ( $P=0.01$ ), and expression of EGFR ( $P=0.03$ ) were associated with responsiveness in patients with samples that underwent immunohistochemical analysis and that never having smoked ( $P<0.001$ ), adenocarcinoma ( $P=0.02$ ), and polysomy or amplification of EGFR ( $P=0.04$ ) were associated with responsiveness in patients with samples subjected to FISH.

#### SURVIVAL

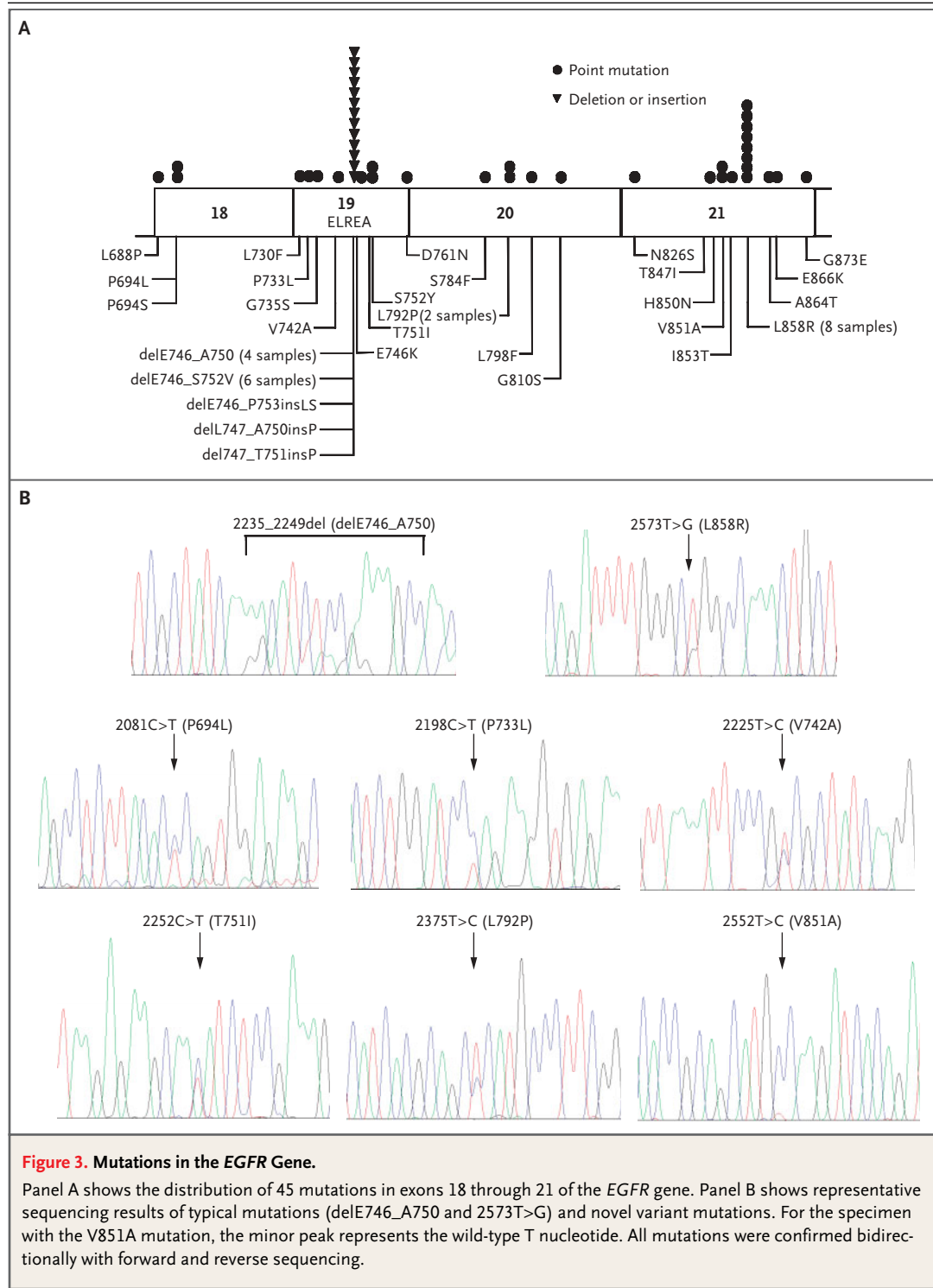
Among the patients in both the placebo and erlotinib groups who had at least one EGFR test, the status of EGFR protein expression, the number of copies of EGFR, and EGFR mutational status were not significantly associated with survival in multivariate analysis, nor were there significant interactions between treatment groups with respect to the status of protein expression ( $P=0.25$ ), the number of copies of EGFR ( $P=0.10$ ), or mutational status ( $P=0.97$ ). Survival among patients with expression of EGFR was longer in the erlotinib group than in the placebo group (hazard ratio for death, 0.68; 95 percent confidence interval, 0.49 to 0.95;  $P=0.02$ ) (Table 3 and Fig. 1D), but there was no survival advantage among patients with EGFR-negative tumors (hazard ratio, 0.93; 95 percent confidence interval, 0.63 to 1.36;  $P=0.70$ ) (Table 3 and Fig. 1C).

Among patients with polysomy or amplification of EGFR, survival was significantly longer among those who received erlotinib than among those who received placebo (hazard ratio for death, 0.44; 95 percent confidence interval, 0.23 to 0.82;  $P=0.008$ ) (Table 3 and Fig. 1F), but there was no significant difference in the length of survival between groups

Variable	Value
<b>Table 2. Summary of the Results of EGFR Analyses and Characteristics of the Patients with EGFR Mutations in Tumor Specimens.</b>	
<b>Immunohistochemical analysis of EGFR protein expression — no. of samples (%)</b>	325
Negative (<10% of tumor cells positive for membranous staining)	141 (43)
Positive ( $\geq 10\%$ of tumor cells positive for membranous staining)	184 (57)
<b>FISH to determine no. of copies of EGFR — no. of samples (%)</b>	125
Disomy ( $\leq 2$ gene copies in >90% of cells)	13 (10)
Low trisomy (3 gene copies in 10–39% of cells)	23 (18)
High trisomy (3 gene copies in $\geq 40\%$ of cells)	3 (2)
Low polysomy ( $\geq 4$ gene copies in 11–39% of cells)	30 (24)
High polysomy ( $\geq 4$ gene copies in $\geq 40\%$ of cells)	42 (34)
Amplification (gene:chromosome $\geq 2$ or $\geq 15$ gene copies per cell in $\geq 10\%$ of cells)	14 (11)
<b>Sequencing to identify EGFR mutations*</b>	177
Wild type (no deletions or mutations detected in exons 18–21) — no. (%)	137 (77)
Mutations — no. (%)	40 (23)
Type of mutation — no. of mutations (%)	45
Exon 19 deletion	13 (29)
Exon 21 L858R	8 (18)
Other variant	24 (53)
<b>Characteristics of the patients with EGFR mutations</b>	
Geographic region — no. of patients/total no. (%)	
North America	12/56 (21)
South America	18/78 (23)
Australia or New Zealand	1/9 (11)
Asia	5/14 (36)
Continental Europe	4/20 (20)
Race or ethnic group — no. of patients/total no. (%)	
Asian	6/12 (50)
Other	34/165 (21)

\* A total of 45 mutations were identified in 40 patients.

among patients with FISH-negative tumors (hazard ratio, 0.85; 95 percent confidence interval, 0.48 to 1.51;  $P=0.59$ ) (Table 3 and Fig. 1E). Mutational status had no significant effect on survival. The risk of death did not differ significantly among patients with EGFR mutations (even Asian patients) who received erlotinib, as compared with such patients who received placebo (hazard ratio for death, 0.77; 95 percent confidence interval, 0.40 to 1.50;  $P=0.54$ ) (Fig. 1H), or among patients with wild-type EGFR



who received erlotinib, as compared with such patients who received placebo (hazard ratio for death, 0.73; 95 percent confidence interval, 0.49 to 1.10;  $P=0.13$ ) (Fig. 1G).

Twenty-one mutations (in 20 patients) were previously described deletions in exon 19 or the L858R mutation in exon 21. There was no significant difference in survival associated with erlotinib thera-

**Table 3. Analysis of Responses and Survival.\***

Factor	No. of Patients	No. Who Could Be Evaluated	No. of Responses (%)	P Value	Hazard Ratio for Death (95% CI)†	P Value
Entire study group	731	427	38 (9)	—	0.70 (0.58–0.85)	<0.001
Sex				0.007		
Male	475	281	17 (6)		0.76 (0.62–0.94)	0.01
Female	256	146	21 (14)		0.80 (0.59–1.07)	0.13
Histopathological subtype				<0.001		
Adenocarcinoma	365	209	29 (14)		0.72 (0.56–0.92)	0.008
Other	366	218	9 (4)		0.81 (0.64–1.02)	0.07
Smoking history				<0.001		
Current or former	545	311	12 (4)		0.87 (0.71–1.05)	0.14
None	146	93	23 (25)		0.42 (0.28–0.64)	<0.001
Unknown	40	23	3 (13)		1.09 (0.54–2.22)	0.80
Race or ethnic group				0.02		
Asian	91	53	10 (19)		0.61 (0.37–1.03)	0.06
Other	640	374	28 (7)		0.79 (0.66–0.95)	0.01
Results of EGFR IHC analysis	325			0.1		
Positive‡	184	106	12 (11)		0.68 (0.49–0.95)	0.02
Negative	141	80	3 (4)		0.93 (0.63–1.36)	0.70
FISH status	125			0.03		
Not amplified	69	41	1 (2)		0.85 (0.48–1.51)	0.59
Amplified§	56	25	5 (20)		0.44 (0.23–0.82)	0.008
Mutational status	170			0.37		
Wild type	137	81	6 (7)		0.73 (0.49–1.10)	0.13
Mutation	40	19	3 (16)		0.77 (0.40–1.50)	0.45

\* Response was assessed according to the Response Criteria in Solid Tumors (RECIST criteria)<sup>34</sup> in patients who had one or more lesions that could be evaluated and at least one follow-up radiologic examination. CI denotes confidence interval, and IHC immunohistochemical.

† The comparison group is the placebo group.

‡ A positive result was one in which 10 percent or more of cells had membranous staining.

§ Results include findings of high polysomy and amplification, as defined in Table 2.

py, as compared with placebo, among patients with the classic exon 19 deletions or the exon 21 L858R mutation (hazard ratio for death, 0.65; 95 percent confidence interval, 0.24 to 1.75;  $P=0.39$ ) and those with only novel mutations (hazard ratio, 0.67; 95 percent confidence interval, 0.26 to 1.75;  $P=0.41$ ) (Fig. 1 of the Supplementary Appendix).

In multivariate Cox regression analysis, treatment with erlotinib, as compared with placebo, remained significantly associated with longer survival ( $P=0.001$ ). In the entire group, Asian patients ( $P=0.01$ ), patients who had never smoked ( $P<0.001$ ), patients who lost less than 5 percent of their body weight ( $P=0.03$ ), patients with a perfor-

mance status of 0 or 1 ( $P<0.001$ ), patients who had not previously received cisplatin ( $P=0.04$ ), and patients who enrolled in the study more than 12 months after receiving a diagnosis of non-small-cell lung cancer ( $P<0.001$ ) survived longest.

## DISCUSSION

Phase 2 studies have shown that female sex, adenocarcinoma, Asian origin, and never having smoked are associated with responsiveness of non-small-cell lung cancer to erlotinib or gefitinib.<sup>16–18,35–37</sup> We confirmed these associations.

The expression of EGFR protein on immuno-

histochemistry has not been a reliable predictor of responsiveness in most studies of EGFR inhibitors.<sup>18,37,38</sup> In our trial, 57 percent of the patients who were tested had tumors that expressed EGFR, and on multivariate analysis, their response rate was higher than that of patients with EGFR-negative tumors (11 percent vs. 4 percent). The expression of EGFR is often associated with polysomy or amplification of *EGFR*.<sup>12</sup> We found that the response rate was significantly higher among patients with tumors with high polysomy or amplification of *EGFR* than among those without this characteristic (20 percent vs. 2 percent). Cappuzzo et al.<sup>22</sup> found that an increased number of copies of *EGFR* was a stronger predictor of response than was the expression of EGFR.

Several groups have found mutations in the *EGFR* tyrosine kinase domain (exons 18 through 21) that sensitize tumor cells to the effects of erlotinib or gefitinib and appear to be associated with responsiveness to these drugs.<sup>19-30</sup> The reported mutations are in-frame deletions, with or without insertions in exon 19, and missense point mutations, mainly in exon 21. The prevalence of mutations varies, ranging from 20 to 40 percent in Asian countries (Taiwan, Korea, and Japan)<sup>23,24,26-28</sup> and from 5 to 19 percent in Italy.<sup>22,25</sup> We identified 45 mutations (13 deletions and 32 point mutations) in 40 patients. The 23 percent prevalence of mutations in our population (40 of 177 patients) is similar to that reported by Shigematsu et al., who analyzed 617 tumors from patients from various regions of the world and found the highest rates among Asian patients.<sup>27</sup> The most common mutations in their study were short deletions in exon 19, corresponding to the region between amino acids 746 and 753 in *EGFR*, and the exon 21 L858R mutation. They also identified point mutations that had not previously been reported. Huang et al.<sup>24</sup> reported 10 new mutations among 117 tumors; 5 were found in 16 tumors that had been embedded in paraffin. Among the approximately 2400 reported analyses for *EGFR* mutations,<sup>19-28</sup> only about 10 percent were performed in formalin-fixed paraffin-embedded specimens, and few routinely used microdissection to increase the number of tumor cells in a given sample.<sup>22,24</sup> The routine application of microdissection to enrich tumor-cell DNA may increase the rate of detection of new mutations. Multiple (two or three) mutations have been identified in individual tumors.<sup>21,23,24,27,29</sup>

In our study, 21 mutations were either dele-

tions in exon 19 or the exon 21 L858R mutation and 24 were novel mutations. In keeping with our results, increasing numbers of novel mutations are being reported in lung cancers and other types of tumors.<sup>23,24,39,40</sup> Among the 24 novel mutations we identified, 1 involves a previously reported codon (V851) but a change in a different amino acid (V851A rather than V851I),<sup>22</sup> and several have also been identified recently by other groups<sup>39</sup> (and unpublished data). In 21 of our patients with such mutations, sufficient DNA was available for reanalysis, and thus, we used independent PCR to confirm the novel sequence in one specimen (D761N). No information on the functional significance of these mutations is available, and none of these mutations have been reported as polymorphisms. A more important point is that no significant difference in survival was associated with erlotinib therapy, as compared with placebo, among patients with the classic exon 19 deletions or the exon 21 L858R mutation (hazard ratio for death, 0.65; 95 percent confidence interval, 0.24 to 1.75;  $P=0.39$ ) and those with only novel mutations (hazard ratio, 0.67; 95 percent confidence interval, 0.26 to 1.75;  $P=0.41$ ) (Fig. 1 of the Supplementary Appendix).

The clinical characteristics of our patients with mutations were similar to those of patients in published studies, with a preponderance of female patients, patients with adenocarcinoma, nonsmoking patients, and Asian patients,<sup>19-28</sup> but we also identified mutations in other subgroups of patients. As in other reports, in our study, the response rate among patients with mutations was more than twice that among patients with wild-type *EGFR*, although the difference was not significant, perhaps because the number of responses was small. The presence of a mutation was not more likely to be associated with responsiveness than were other clinical characteristics.

A meta-analysis<sup>14</sup> has suggested that in patients with non-small-cell lung cancer who are not receiving EGFR-inhibitor therapy, expression of EGFR is not a strong prognostic factor for survival. Our findings were similar, but whether the expression of EGFR is associated with responsiveness to erlotinib or to a differential effect of erlotinib on survival requires further exploration.

Cappuzzo et al.<sup>22</sup> reported that only the number of copies of *EGFR* was significantly related to survival in a multivariate analysis of patients who were treated with gefitinib. In our study, however,

the number of copies of *EGFR* was not a significant prognostic factor in multivariate analysis.

The view that patients with wild-type tumors would not benefit from treatment with EGFR inhibitors and thus should not receive these agents was reinforced by reports that patients with *EGFR* mutations in their tumor cells survived longer than patients without such mutations.<sup>26,28,30</sup> However, none of these studies included an untreated control group, and thus, they were unable to determine whether this finding was due to a differential effect of treatment on tumors with mutations or to the indolent behavior of tumors with mutations. Although there was a higher rate of response among the small number of patients with mutations than among those with wild-type *EGFR* in our study, the presence of a mutation was not associated with the survival benefit of erlotinib therapy. The benefit from erlotinib, as compared with placebo, among patients with wild-type *EGFR* (hazard ratio for death,

0.73) was similar to that observed in the population as a whole (hazard ratio, 0.70).

In summary, multivariate analysis revealed that expression of *EGFR* and an increased number of copies of *EGFR*, but not mutations in *EGFR*, were associated with responsiveness to erlotinib but not with increased survival. Our results suggest that mutational analysis is not necessary to identify patients in whom treatment with EGFR inhibitors is appropriate.

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#### REFERENCES

- Salomon D, Gullick W. The erbB family of receptors and their ligands: multiple targets for therapy. *Signal* 2001;2:4-11.
- Schlessinger J. Ligand-induced, receptor-mediated dimerization and activation of EGF receptor. *Cell* 2002;110:669-72.
- Arteaga CL. Overview of epidermal growth factor receptor biology and its role as a therapeutic target in human neoplasia. *Semin Oncol* 2002;29:Suppl 14:3-9.
- Herbst RS, Bunn PA Jr. Targeting the epidermal growth factor receptor in non-small cell lung cancer. *Clin Cancer Res* 2003;9:5813-24.
- Nicholson RI, Gee JMW, Harper ME. EGFR and cancer prognosis. *Eur J Cancer* 2001;37:Suppl 4:S9-S15.
- Veale D, Kerr N, Gibson GJ, Kelly PJ, Harris AL. The relationship of quantitative epidermal growth factor receptor expression in non-small cell lung cancer to long term survival. *Br J Cancer* 1993;68:162-5.
- Rusch V, Klimstra D, Venkatraman E, Pisters PW, Langenfeld J, Dmitrovsky E. Overexpression of the epidermal growth factor receptor and its ligand transforming growth factor alpha is frequent in resectable non-small cell lung cancer but does not predict tumor progression. *Clin Cancer Res* 1997;3:515-22.
- Fontanini G, De Laurentiis M, Vignati S, et al. Evaluation of epidermal growth factor-related growth factors and receptors and of neoangiogenesis in completely resected stage I-III non-small-cell lung cancer: amphiregulin and microvessel count are independent prognostic indicators of survival. *Clin Cancer Res* 1998;4:241-9.
- Hsieh ET, Shepherd FA, Tsao M-S. Co-expression of epidermal growth factor receptor and transforming growth factor- $\alpha$  is independent of ras mutations in lung adenocarcinoma. *Lung Cancer* 2000;29:151-7.
- Brabender J, Danenberg KD, Metzger R, et al. Epidermal growth factor receptor and HER2-neu mRNA expression in non-small cell lung cancer is correlated with survival. *Clin Cancer Res* 2001;7:1850-5.
- Selvaggi G, Novello S, Torri V, et al. Epidermal growth factor receptor overexpression correlates with a poor prognosis in completely resected non-small-cell lung cancer. *Ann Oncol* 2004;15:28-32.
- Hirsch FR, Varella-Garcia M, Bunn PA Jr, et al. Epidermal growth factor receptor in non-small-cell lung carcinomas: correlation between gene copy number and protein expression and impact on prognosis. *J Clin Oncol* 2003;21:3798-807.
- Swinson DE, Cox G, O'Byrne KJ. Coexpression of epidermal growth factor receptor with related factors is associated with a poor prognosis in non-small-cell lung cancer. *Br J Cancer* 2004;91:1301-7.
- Meert A-P, Martin B, Delmotte P, et al. The role of EGF-R expression on patient survival in lung cancer: a systematic review with meta-analysis. *Eur Respir J* 2002;20:975-81.
- Sridhar SS, Seymour L, Shepherd FA. Inhibitors of epidermal-growth-factor receptors: a review of clinical research in patients with a focus on non-small-cell lung cancer. *Lancet Oncol* 2003;4:397-406.
- Fukuoka M, Yano S, Giaccone G, et al. Multi-institutional randomized phase II trial of gefitinib for previously treated patients with advanced non-small cell lung cancer. *J Clin Oncol* 2003;21:2237-46. [Erratum, *J Clin Oncol* 2004;22:4811.]
- Kris MG, Natale RB, Herbst RS, et al. Efficacy of gefitinib, an inhibitor of the epidermal growth factor receptor tyrosine kinase, in symptomatic patients with non-small cell lung cancer: a randomized trial. *JAMA* 2003;290:2149-58.
- Pérez-Soler R, Chachoua A, Hammond LA, et al. Determinants of tumor response and survival with erlotinib in patients with non-small-cell lung cancer. *J Clin Oncol* 2004;22:3238-47.
- Lynch TJ, Bell DW, Sordella R, et al. Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. *N Engl J Med* 2004;350:2129-39.
- Paez JG, Janne PA, Lee JC, et al. EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. *Science* 2004;304:1497-500.
- Pao W, Miller V, Zakowski M, et al. EGF receptor gene mutations are common in lung cancers from "never smokers" and are associated with sensitivity of tumors to gefitinib and erlotinib. *Proc Natl Acad Sci U S A* 2004;101:13306-11.
- Cappuzzo F, Hirsch F, Rossi E, et al. Epidermal growth factor receptor gene and protein and gefitinib sensitivity in non-small cell lung cancer. *J Natl Cancer Inst* 2005;97:643-55.
- Kosaka T, Yatabe Y, Endoh H, Kuwano H, Takahashi T, Mitsudomi T. Mutations of the epidermal growth factor receptor gene in lung cancer: biological and clinical implications. *Cancer Res* 2004;64:8919-23.
- Huang S-F, Liu H-P, Li L-H, et al. High

- frequency of epidermal growth factor receptor mutations with complex patterns in non-small cell lung cancers related to gefitinib responsiveness in Taiwan. *Clin Cancer Res* 2004;10:8195-203.
25. Marchetti A, Martella C, Felicioni L, et al. EGFR mutations in non-small-cell lung cancer: analysis of a large series of cases and development of a rapid and sensitive method for diagnostic screening with potential implications on pharmacologic treatment. *J Clin Oncol* 2005;23:857-65.
26. Han S-W, Kim T-Y, Hwang P-G, et al. Predictive and prognostic impact of epidermal growth factor receptor mutation in non-small-cell lung cancer patients treated with gefitinib. *J Clin Oncol* 2005;23:2493-501.
27. Shigematsu H, Lin L, Takahashi T, et al. Clinical and biological features associated with epidermal growth factor receptor gene mutations in lung cancers. *J Natl Cancer Inst* 2005;97:339-46.
28. Tokumo M, Toyooka S, Kiura K, et al. The relationship between epidermal growth factor receptor mutations and clinicopathologic features in non-small cell lung cancers. *Clin Cancer Res* 2005;11:1167-73.
29. Yang SH, Mechanic LE, Yang P, et al. Mutations in the tyrosine kinase domain of the epidermal growth factor receptor in non-small cell lung cancer. *Clin Cancer Res* 2005;11:2106-10.
30. Kim KS, Jeong JY, Kim YC, et al. Predictors of the response to gefitinib in refractory non-small cell lung cancer. *Clin Cancer Res* 2005;11:2244-51.
31. Pedersen MW, Meltorn M, Damstrup L, Poulsen HS. The type III epidermal growth factor receptor mutation: biological significance and potential target for anti-cancer therapy. *Ann Oncol* 2001;12:745-60.
32. Stephens P, Hunter C, Bignell G, et al. Lung cancer: intragenic ERBB2 kinase mutations in tumours. *Nature* 2004;431:525-6.
33. Shepherd FA, Pereira JR, Ciuleanu T, et al. Erlotinib in previously treated non-small-cell lung cancer. *N Engl J Med* 2005;353:123-32.
34. Therasse P, Arbuck SG, Eisenhauer EA, et al. New guidelines to evaluate the response to treatment in solid tumors. *J Natl Cancer Inst* 2000;92:205-16.
35. Miller VA, Kris MG, Shah N, et al. Bronchioloalveolar pathologic subtype and smoking history predict sensitivity to gefitinib in advanced non-small-cell lung cancer. *J Clin Oncol* 2004;22:1103-9.
36. Janne PA, Gurubhagavatula S, Yeap BY, et al. Outcomes of patients with advanced non-small cell lung cancer treated with gefitinib (ZD1839, "Iressa") on an expanded access study. *Lung Cancer* 2004;44:221-30.
37. Dancey JE. Predictive factors for epidermal growth factor receptor inhibitors — the bull's-eye hits the arrow. *Cancer Cell* 2004;5:411-5.
38. Parra HS, Cavina R, Latteri F, et al. Analysis of epidermal growth factor receptor expression as a predictive factor for response to gefitinib ("Iressa", ZD1839) in non-small-cell lung cancer. *Br J Cancer* 2004;91:208-12.
39. Weber F, Fukino K, Sawada T, et al. Variability in organ-specific EGFR mutational spectra in tumour epithelium and stroma may be the biological basis for differential responses to tyrosine kinase inhibitors. *Br J Cancer* 2005;92:1922-6.
40. Nagahara H, Mimori K, Ohta M, et al. Somatic mutations of epidermal growth factor receptor in colorectal carcinoma. *Clin Cancer Res* 2005;11:1368-71.

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**CORRECTION**

**Erlotinib in Lung Cancer — Molecular and Clinical Predictors of Outcome**

Erlotinib in Lung Cancer — Molecular and Clinical Predictors of Outcome . On page 139, the second line from the bottom of the right-hand column should have read "95 percent confidence interval, 0.40 to 1.50; P=0.45)," not "P=0.54," as printed.