Exon 19 Deletion Mutations of Epidermal Growth Factor Receptor Are Associated with Prolonged Survival in Non–Small Cell Lung Cancer Patients Treated with Gefitinib or Erlotinib

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Abstract

Purpose: Somatic mutations in the epidermal growth factor receptor (EGFR) have been detected in patients with non–small cell lung cancer (NSCLC) and are associated with sensitivity to treatment with gefitinib or erlotinib. Our study explored the relationship between the two most common types of somatic EGFR mutations, exon 19 deletions and the L858R point mutation, and outcomes of patients following treatment with gefitinib or erlotinib.

Experimental Design: Tumor specimens obtained before treatment with gefitinib or erlotinib were analyzed for EGFR mutations. Patients with exon 19 deletion or L858R mutations were identified. The response rate, time to progression, and overall survival were determined for the two groups.

Results: We identified 36 patients with NSCLC and an EGFR mutation who were treated with gefitinib or erlotinib. Patients with an exon 19 deletion had a significantly longer overall survival compared with patients with an L858R mutation (38 versus 17 months; $P = 0.04$). There were also trends toward higher response rate (73% versus 50%) and improved time to progression (24 versus 10 months) for the patients with an exon 19 deletion, although these were not independently significant in a multivariate analysis. A difference in response rate for patients treated with gefitinib compared with erlotinib was also noted [18 of 23 (78%) versus 3 of 9 (33%); $P = 0.04$]. No obvious difference in time to progression or overall survival was noted between gefitinib- and erlotinib-treated patients.

Conclusions: Patients with NSCLC and EGFR exon 19 deletions have a longer survival following treatment with gefitinib or erlotinib compared with those with the L858R mutation. Pooling of greater numbers of patients and completion of prospective trials are needed to further define the predictive and prognostic roles of different EGFR mutations with respect to treatment with gefitinib, erlotinib, and other EGFR inhibitors.

Lung cancer remains the leading cause of cancer death in the United States, with an estimated 162,000 deaths in 2006 (1). Before the introduction of targeted agents for the treatment of patients with advanced non–small cell lung cancer (NSCLC), standard regimens of platinum-based chemotherapy resulted in a median survival of ~10 months (2). The targeted agents gefitinib and erlotinib are small-molecule inhibitors of the tyrosine kinase domain of the epidermal growth factor receptor (EGFR). EGFR expression can be detected by immunohistochemistry in as many as 57% to 99% of cases of NSCLC (3–5). Although only 8% to 18% of patients with progressive NSCLC after prior chemotherapy will have a clinical response to these agents, the responses can be dramatic and may last for longer than a year (6–10). Laboratory efforts focused on determining

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Note: Drs. Bell, Haber, Jänne, Johnson, Lynch, and Meyerson are part of a pending patent application on EGFR mutation testing.

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the mechanisms of response and resistance to these EGFR inhibitors and defining the subset of patients likely to benefit clinically from treatment with these agents.

Researchers identified somatic mutations in the EGFR in 2004 and discovered that these mutations were associated with a high likelihood of clinical response to treatment with gefitinib and erlotinib (11–13). Although nearly all recently published trials attest to the association between EGFR mutations and sensitivity to treatment with either erlotinib or gefitinib, the clinical response rates and response duration in patients harboring such somatic mutations have varied substantially in the different studies (4, 14–18) Some of the variation between mutation and outcome may be caused by underlying differences in the types of EGFR somatic mutations identified and the efficacy of gefitinib or erlotinib against those mutated receptors.

Patients with gastrointestinal stromal tumors provide an example of the effect of tumor genotype on treatment outcome. In this disease, a multicenter effort analyzed 127 tumor specimens for different mutations in the KIT receptor and in the platelet-derived growth factor receptor-α. Following treatment with imatinib, patients with exon 11 KIT somatic mutations experienced a 2-fold increase in response rate and a significant prolongation in survival compared with those with an exon 9 KIT mutation (19).

A similar analysis of EGFR genotype in patients with NSCLC and outcome following treatment with gefitinib and erlotinib is warranted. To date, EGFR somatic mutations are associated with either sensitivity (11–13) or resistance (20–22) to gefitinib and erlotinib treatment. The two most common EGFR somatic mutations, exon 19 deletions and L858R missense mutations, have been associated with *in vitro* and *in vivo* sensitivity to treatment with the EGFR tyrosine kinase inhibitors (EGFR-TKI) gefitinib and erlotinib. These two different types of mutations are responsible for ~85% of all EGFR somatic mutations identified in patients with NSCLC (23). Lung cancer cell lines harboring these two different classes of mutations have shown similar sensitivity to gefitinib as measured by 72-hour growth inhibition studies. The cell line NCI-H3255, which harbors the L858R mutation, has a gefitinib IC_{50} of 40 to 63 nmol/L (12, 24), whereas the cell lines DFCILU-011 (del L747_E749) and PC-9 (del E746_A750) have a IC_{50} of approximately 10 and 20 nmol/L, respectively (24). However, such short-term studies may not be reflective of long-term exposure to gefitinib or erlotinib that occurs over several months or even years in patients with NSCLC.

In the present study, we identified patients whose tumor had a detectable EGFR somatic mutation and who were treated with gefitinib or erlotinib. The aim of the study was to correlate clinical response to gefitinib or erlotinib, time to progression, and survival with EGFR tumor genotype. The analysis was specifically focused on patients with one of the two most common EGFR mutations: a deletion in exon 19 or an L858R point mutation. By focusing the analysis on these two specific mutations, we were able to collect data on sufficient numbers of patients to allow for a comparative analysis.

Materials and Methods

Patients. This retrospective analysis comprises 36 patients with advanced NSCLC with a somatic mutation of their tumor EGFR who were treated with either gefitinib or erlotinib. To be eligible for inclusion in this study, patients had to have histologically or cytologically confirmed NSCLC (stage IIIB or IV), signed consent allowing for correlative studies of their tumor (including sequencing of the DNA from the tumor), documented presence of an EGFR mutation, and treatment with either gefitinib or erlotinib. Patients who were started on treatment with gefitinib or erlotinib from January 2001 to March 2005 were included in this study. Those who started on treatment with an EGFR-TKI after March 2005 were excluded to assure a minimum follow-up of at least 1 year.

The patients included in this study represent all of the patients from our institution who met the above eligibility criteria and were started on treatment with gefitinib or erlotinib before March 2005. Patients were identified through a query of an internal database within the Lowe Center for Thoracic Oncology at the Dana-Farber Cancer Institute (Boston, MA) that tracks all of the patients referred for EGFR sequencing from our center. Additional EGFR mutation-positive patients who had met all of the above eligibility criteria were supplied by Beth Israel Deaconess Medical Center (Boston, MA), a member of the Dana-Farber/Harvard Cancer Center. In addition, patients who were part of our original publications describing EGFR mutations were included (11, 12).

Nine of the patients included in this analysis were treated with erlotinib as part of a phase II trial for elderly patients with previously untreated advanced NSCLC (25). Nine patients were treated with gefitinib as part of the Iressa Expanded Access Program. The remaining 18 patients were treated with gefitinib after its Food and Drug Administration approval in May 2003. Some of the patients in this study have been included in earlier publications from investigators within the Dana-Farber/Harvard Cancer Center (11, 12, 20, 24, 26, 27).

Mutation analysis. Tumor specimens for each patient on this study were obtained from diagnostic or surgical procedures. Samples consisted of either frozen tumor specimens or paraffin-embedded material. For patients with sufficient amounts of tissue to undergo analyses by direct DNA sequencing, tumor cells were isolated from the normal cells by microdissection so that >50% tumor cells were present in the specimen sent for sequencing. Exons 18 to 24 were amplified by PCR and analyzed bidirectionally by direct sequencing for the presence of somatic mutations according to previously described methods (11, 12, 28). All mutations were confirmed by multiple independent PCR amplifications.

An alternate method of mutation screening was used for those tumor samples that were deemed inadequate for direct sequencing based on review by a molecular pathologist (N.L.) and/or specimens with a high percentage of normal cells (<50% tumor cells; ref. 26). Formalin-fixed, paraffin-embedded tissues were obtained either as a set of ten 5-μm slides or as uncut tissue blocks. Excess paraffin was removed, and DNA was extracted using Qiagen DNeasy Tissue kit (Valencia, CA) with previously described modifications (29). Exons 18 to 21 were amplified by PCR; the PCR products were then analyzed by digestion with SURVEYOR, a DNA endonuclease that cleaves mismatched heteroduplexed DNA. The resulting fragments were then analyzed by high-performance liquid chromatography on the Transgenomic WAVE Nucleic Acid High Sensitivity Fragment Analysis System (Transgenomic, Inc., Omaha, NE). All mutations detected by the WAVE Nucleic Acid High Sensitivity Fragment Analysis System were confirmed by repeat analysis to prove that the signal observed on the SURVEYOR indeed corresponded to a mutation. The mutant allele was enriched by denaturing high-performance liquid chromatography followed by fractionation and sequencing as described previously (26).

Statistical methods. Baseline clinical characteristics were determined by retrospective chart review, including age at diagnosis, gender, race, baseline Eastern Cooperative Oncology Group performance status at the start of treatment with an EGFR-TKI, smoking status, tumor histology, the number of prior chemotherapy regimens received, and the EGFR-TKI administered (gefitinib versus erlotinib). Smoking status was categorized as current, former (quit ≥1 year before diagnosis), or

never (<100 lifetime cigarettes). Tumor histology was classified using WHO criteria (30). Fisher's exact test was used to compare the distribution of categorical characteristics between mutation types, whereas Wilcoxon rank-sum test was used to compare the age distribution.

Best clinical response to treatment with gefitinib or erlotinib was classified based on interval computed tomography scans as complete response, partial response, stable disease, or progressive disease using standard Response Evaluation Criteria in Solid Tumors criteria (31). Tumor response rates were calculated for each type of EGFR mutation, and response rates between exon 19 deletions and L858R point mutations were compared using Fisher's exact test (32). Time to progression and survival were measured for each patient from the first day treatment with gefitinib or erlotinib was initiated. The outcome was censored if a patient had not progressed or died at the time of last follow-up. Overall time to progression and survival were estimated using the Kaplan-Meier method, with differences between the groups compared using the log-rank test (33).

Logistic and proportional hazards models were fitted to the response, time to progression, and overall survival data to assess the independent effects of mutational status and the baseline clinical characteristics described previously. A stepwise regression procedure was conducted to retain those variables with a $P < 0.1$ (34). Statistical analyses were done using Statistical Analysis System version 9.1 (SAS Institute, Inc., Cary, NC).

Results

Patient characteristics. Between January 2001 and March 2005, 36 patients who were found to have an EGFR mutation in DNA from their tumor specimen were begun on treatment with gefitinib or erlotinib. The location and frequency of these mutations are shown graphically in Fig. 1. The most common type of mutation (22 patients, 61%) was an in-frame deletion in exon 19. All exon 19 deletions are considered as one category in this study. The L858R point mutation on exon 21 was identified in 10 (28%) patients. A small number of other point mutations were discovered, with one patient having both a G719S and L861Q mutation. In addition, 28 of 36 (75%) patients were analyzed for K-*ras* mutations, and no K-*ras* mutations were identified (data not shown).

The primary focus of this analysis is patients with either exon 19 deletions or L858R point mutations. Table 1 shows the baseline characteristics of the patients found to have one of these mutations. Although this study represents a retrospective series, the groups are reasonably balanced with respect to baseline characteristics and other previously identified prognostic variables, with no statistically meaningful differences found between the two groups.

Correlation of EGFR genotype with clinical response to an EGFR-TKI. The best clinical response following treatment with gefitinib or erlotinib was classified as complete response, partial response, stable disease, or progressive disease. The response rates were calculated for both the exon 19 deletion group and the L858R point mutation group (Table 2). There was a slightly higher response rate in patients with exon 19 deletions, although this improvement was not statistically significant. Moreover, there were two complete responses (of 11 and 24 months duration) observed in that group compared with none in the L858R group.

The strongest association of response in these patients with known EGFR mutations was treatment with gefitinib rather than erlotinib. Eighteen of 23 (78%) patients treated with gefitinib had a documented clinical response compared with only 3 of 9 (33%) patients treated with erlotinib ($P = 0.035$). A stepwise logistic regression was then done to identify other clinical factors that might jointly predict response to treatment with an EGFR-TKI in patients with known EGFR mutations. Gefitinib therapy was the only variable noted to predict response in this multivariate analysis, with a hazard ratio of 0.16 ($P = 0.043$), after adjusting for the mutation effect.

Correlation of EGFR genotype with time to progression. A Kaplan-Meier analysis was used to determine the time to progression for the entire cohort of patients with NSCLC and EGFR mutations who were treated with gefitinib or erlotinib. With a median potential follow-up of 24 months, 25 (78%) patients had developed clinical progression: 16 (73%) in the exon 19 deletion group and 9 (90%) in the L858R group. The median time to progression for the whole group was 13 months (Fig. 2A).

The time to progression was then analyzed with respect to EGFR genotype, comparing patients with exon 19 deletions with those with L858R point mutations (Fig. 2B). For patients with an in-frame deletion of exon 19, the median time to

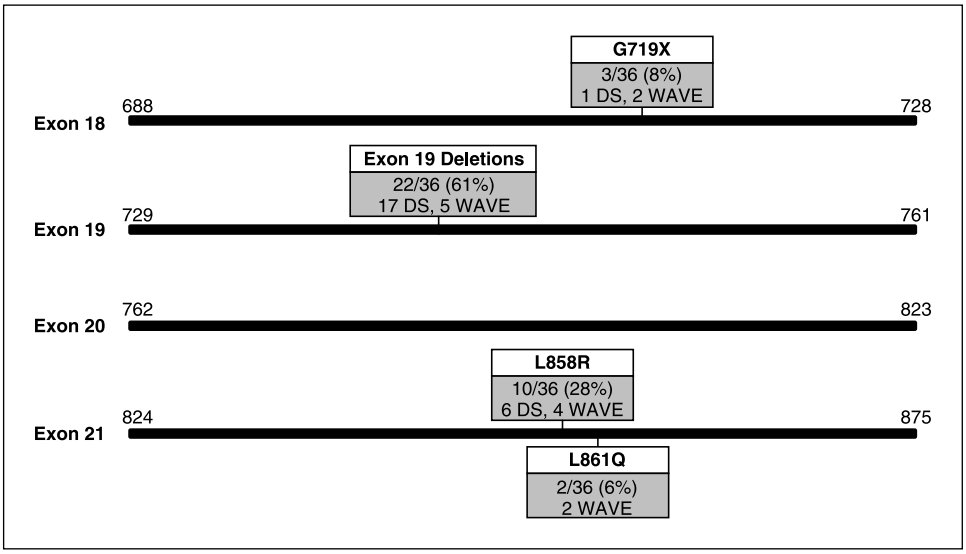

Fig. 1. Location and frequency of EGFR mutations identified. Horizontal bars, exons 18 to 21 of the tyrosine kinase domain of the EGFR, with the corresponding codons for each exon (*left* and *right*). Box, each specific type of somatic mutation. Gray portion, proportion and percentage for which a specific mutation accounted out of the total 36 mutations found; also listed is the method used to detect these mutations. DS, direct sequencing; WAVE, Transgenomic WAVE Nucleic Acid High Sensitivity Fragment Analysis System. Note: one patient had both G719A and L861Q mutations.

Table 1. Baseline characteristics of patients identified with exon 19 deletions or L858R point mutations

	Exon 19 deletions (*N* = 22)	L858R (*N* = 10)	*P*
Sex, *n* (%)			1.00
Male	8 (36)	4 (40)	
Female	14 (64)	6 (60)	
Age, median (range)	65.5 (44-81)	70 (32-79)	0.78
Race, *n* (%)			1.00
White, non-Hispanic	18 (82)	9 (90)	
Hispanic	1 (5)	1 (10)	
Asian	2 (9)	0	
Black	0	0	
Other	1 (5)	0	
Performance status, *n* (%)			0.39 (0-1 vs 2-3)
0	1 (5)	3 (30)	
1	15 (68)	6 (60)	
2	1 (5)	1 (10)	
3	5 (23)	0 (0)	
Smoking history, *n* (%)			1.00
None	10 (45)	5 (50)	
Former	12 (55)	5 (50)	
Current	0	0	
Histology, *n* (%)			0.33
Adeno (non-BAC)	13 (59)	7 (70)	
Adeno with BAC	4 (18)	0	
BAC	4 (18)	1 (10)	
Other	1 (5)	2 (20)	
No. prior chemotherapies, *n* (%)			0.65
0	9 (41)	7 (70)	
1	5 (23)	1 (10)	
2	3 (14)	1 (10)	
3	2 (9)	1 (10)	
4	3 (14)	0	
EGFR-TKI, *n* (%)			0.10
Gefitinib	18 (82)	5 (50)	
Erlotinib	4 (18)	5 (50)	

NOTE: Due to rounding, some cells do not add to 100%.
Abbreviation: BAC, bronchioloalveolar carcinoma.

progression was 24 months compared with 10 months for patients with an L858R mutation ($P = 0.04$). A stepwise Cox proportional hazards analysis was done to evaluate for a correlation between progression and the clinical characteristics described previously. Although bronchioloalveolar carcinoma histology ($P = 0.01$) and female gender ($P = 0.026$) were correlated simultaneously with a longer time to progression, the EGFR genotype did not independently predict for time to progression.

Correlation of EGFR genotype with overall survival. At the time of this analysis, 14 (44%) deaths had occurred: 8 in those with exon 19 deletions and 6 with L858R mutations. The median survival for the whole group was 30 months (Fig. 2C). When survival was compared by EGFR genotype, patients whose NSCLC DNA had a detectable exon 19 deletion had a statistically significant prolongation in survival, with a median survival of 38 months compared with 17 months for patients with an L858R point mutation ($P = 0.0384$; Fig. 2D). Again, a stepwise proportional hazards model for survival was fitted with the potential prognostic factors described previously. The presence of an exon 19 deletion rather than the L858R point mutation was the strongest prognostic factor, with a hazard ratio of 0.20 ($P = 0.013$). The only other variable that was correlated independently to improved survival was good baseline performance status (Eastern Cooperative Oncology Group performance status of 0 or 1) at the time of initiation of treatment with gefitinib or erlotinib (hazard ratio, 0.23; $P = 0.027$). Although both gender and tumor histology had been correlated to improved time to progression, neither of these clinical characteristics was correlated with survival. Moreover, although the specific EGFR inhibitor (gefitinib versus erlotinib) was correlated to response, there was no relationship found between the EGFR inhibitor used and time to progression and overall survival.

Discussion

In the current study, we report on differences in outcome based on EGFR genotype after treatment with gefitinib or erlotinib in patients with NSCLC. A total of 36 eligible patients with EGFR mutations were identified. Of these, 32 (89%) patients had either an exon 19 deletion ($n = 22$) or the L858R point mutation ($n = 10$). This is the second study to compare patients with exon 19 deletions with those harboring an L858R point mutation and provides important independent validation to earlier observations (35). In a previously reported study, Riely et al. analyzed 34 NSCLC patients with either an exon 19 deletion ($n = 23$) or an L858R mutation ($n = 11$). Of these, 22 were treated with gefitinib and 12 were treated with erlotinib. The baseline characteristics of the patients in both studies are remarkably similar with respect to age,

Table 2. Response to gefitinib or erlotinib in NSCLC patients with exon 19 deletions or L858R point mutations

Response	Exon 19 deletion (*N* = 22)	L858R mutation (*N* = 10)	*P*
Best response			
CR	2	0	
PR	14	5	
SD	6	5	
PD	0	0	
Response rate	73%	50%	0.25
Disease control rate	100%	100%	1.00
Median duration of treatment (mo)			
CR/PR	16	13	0.46
SD	26	10	0.01

Abbreviations: CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease.

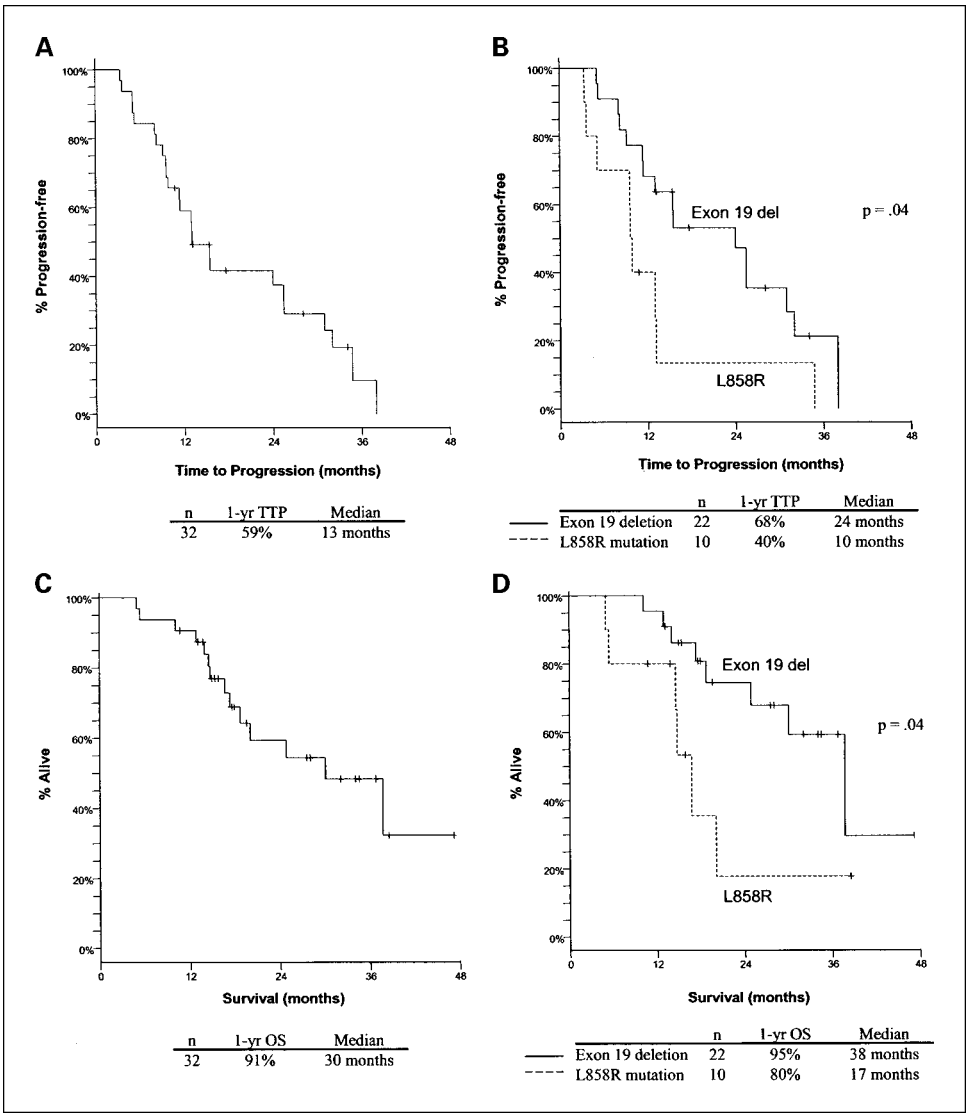

Fig. 2. Time to progression and overall survival based on EGFR genotype. *A,* overall time to progression (*TTP*). *B,* time to progression in patients with exon 19 deletions versus L858R mutations. *C,* overall survival (*OS*). *D,* survival duration in patients with exon 19 deletions versus L858R mutations.

gender, smoking status, tumor histology, performance status, number of prior chemotherapy regimens, and EGFR-TKI administered.

Although the radiographic response rate in our study was higher in patients with exon 19 deletion mutations compared with those with L858R, these findings were not statistically different (Table 2). These response rates are similar to those reported by Cappuzzo et al. (4), who found five responses in seven patients with exon 19 deletions (response rate, 71%) compared with only three responses in seven patients with an L858R mutation (response rate, 43%). Mitsudomi et al. also found a correlation between EGFR genotype and response rate. In that study, 16 of 16 (100%) patients with an exon 19 deletion showed a clinical response, whereas 8 of 12 (67%) patients with a point mutation responded; however, it should be noted that the latter group included all point mutations and not just the L858R mutation (16). In the series from Riely et al. (35), response rates for the two mutation groups were not specifically reported.

In our study, the time to progression and survival for patients with NSCLC and somatic exon 19 deletions were prolonged compared with L858R mutations (Fig. 2). These data support similar results reported by Riely et al. (35), who also found a survival advantage in favor of exon 19 deletions over L858R mutations (median of 34 versus 8 months; P = 0.01). To determine whether EGFR genotype was independently predictive of time to progression and survival, we did a stepwise regression analysis. Although EGFR genotype was not shown to be an independent predictor of time to progression, there was a strong correlation between EGFR genotype and survival. This survival benefit was independent on other clinical variables, including treatment administered (gefitinib versus erlotinib). These data suggest that it is not only the presence or absence of an EGFR mutation that is important; the specific type of mutation may have additional implications in predicting outcome to treatment with gefitinib or erlotinib. The work done in patients with gastrointestinal stromal tumors, which has an annual incidence of ~4,300 cases, showed that tumor genotype might play an important role in predicting treatment with the TKI imatinib (19, 36). Similar findings would have an even broader effect in NSCLC, where EGFR mutations are found in ~10% of cases or 17,000 patients annually.

Although patients with NSCLC and exon 19 deletions who are treated with gefitinib or erlotinib live longer than those with an L858R mutation, it is possible that the mutation itself could have an effect on outcome. This has been studied in patients with surgically resected early-stage NSCLC who were not treated with gefitinib or erlotinib (23). Here, the 31 patients with surgically resected NSCLC and an L858R mutation had a longer overall survival (median survival in excess of 100 months) compared with 31 patients with an exon 19 deletion (median survival ~40 months) and 365 patients without an EGFR mutation (~50 months). Although this difference fell just short of statistical significance (P = 0.06), the data provide some understanding of the potential prognostic significance of different EGFR mutations in patients not treated with gefitinib or erlotinib. The data also suggest that the survival advantage found after treatment with gefitinib or erlotinib in our study and in the study by Riely et al. reflects an even more significant alteration in the potential course of disease for patients with exon 19 deletions (35). However, one cannot be certain that the effect of EGFR mutation on outcomes in patients with surgically resected, early-stage disease will be the same as that observed in patients presenting with advanced NSCLC.

It remains unclear why clinical outcome for patients with NSCLC should differ between exon 19 deletions and L858R point mutations following treatment with gefitinib or erlotinib. One possibility is that exon 19 deletions are more efficiently inhibited by gefitinib or erlotinib than L858R. However, *in vitro* studies do not support this hypothesis. NSCLC cell lines bearing exon 19 deletion or L858R mutations are similarly growth inhibited by gefitinib or erlotinib (12, 24). Furthermore, EGFR phosphorylation is completely inhibited by equivalent concentrations of gefitinib in NIH-3T3 cells expressing either L858R or an exon 19 deletion (24). An alternative hypothesis is that T790M mutations, which have been associated with acquired resistance to EGFR-TKIs (20, 37), might occur more frequently in the presence of an L858R mutation compared with an exon 19 deletion. However, published data are thus far too limited to draw any such conclusion. Of five cases of T790M-associated resistance described in the current literature, three patients harbored an L858R point mutation in their initial tumor sample, whereas two had an exon 19 deletion (20, 37, 38). Continued analyses of tumor specimens from patients who develop acquired resistance to gefitinib or erlotinib are needed to answer this question.

An interesting finding in our study was the difference in response based on the particular EGFR-TKI received. In these patients with known EGFR mutations, gefitinib treatment was associated with a significantly higher response rate than erlotinib (78% versus 33%; P = 0.0354). This difference persisted after multivariate analysis. However, there was no difference in time to progression or overall survival between the gefitinib- and erlotinib-treated patients. The study by Riely et al. involved similar numbers of patients with EGFR mutations who were treated with gefitinib (n = 22) or erlotinib (n = 12), but no specific mention is made about any difference in outcome based on the drug administered (35). Although it is difficult to draw conclusions from our small retrospective study, the response rates reported here are fairly similar to those found in published studies to date. In the six studies that have reported response rates for patients with known EGFR mutations who received gefitinib, response rates varied from 54% to 94%; in total, there were 93 responses found in 122 EGFR mutation-positive patients in these studies, for an overall response rate of 76% (4, 14–18). To date, there has been only one published study that has reported the response rate for patients treated with erlotinib who were known to have an EGFR mutation (9). When analysis of the BR.21 study was revised to include only patients with either exon 19 deletions or an L858R point mutation, there were three responses among 10 patients who were treated with erlotinib (response rate, 30%), similar to our finding of three responses in 9 erlotinib-treated patients (39). Larger numbers of patients and prospective evaluation are required to further explore this difference. There are currently several trials under way that involve prospective treatment of patients with EGFR mutations with gefitinib or erlotinib. Furthermore, there are many other EGFR inhibitors in clinical development and they will continue to be important to correlate response to treatment with different EGFR mutations and different EGFR-targeted agents.

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