Predictors of the Response to Gefitinib in Refractory Non–Small Cell Lung Cancer

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ABSTRACT

Gefitinib, an epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor, has a response rate of 10% to 20% in refractory non–small cell lung carcinoma. Although female gender, adenocarcinoma, and never having smoked are possible markers of a favorable response, mutations of the *EGFR* gene have also been reported to be highly significant predictors of response. Seventy patients with relapsed non–small cell lung carcinoma were enrolled in the Expanded Access Program. After the drug became available commercially, 28 more patients were treated with gefitinib. Response evaluations were feasible in 80 patients. Twenty-seven tumor specimens (8 responders and 19 non-responders) were available for the sequence analysis of the *EGFR* gene. The response rate was 25% (20/80) and the disease control rate (remission + stable disease) was 47.5% (38/80). The response rate was significantly higher for adenocarcinoma (41.0%) versus non-adenocarcinoma (9.8%, $P = 0.001$), in those who never smoked (58.8%) versus smokers (15.9%, $P < 0.001$), and in females (42.1%) versus males (19.7%, $P = 0.049$). A deletion or mutation of the *EGFR* gene was found in six of eight responders. Remission was noted in all patients with a mutation, whereas the response rate was 9.5% (2/21) in patients without a mutation ($P < 0.001$). The predictors of response showed significant correlations with survival and time to progression. In a multivariate logistic analysis, the independent predictors of response were smoking history and adenocarcinoma. Given that 9.5% of smokers and 6.7% of those with non-adenocarcinoma showed a mutation of the *EGFR* gene, the genetic profile may replace those variables as an independent predictor of a response.

INTRODUCTION

Lung cancer has been the leading cause of cancer death in South Korea, as in many other parts of the world, since the year 2000 (1). As the global burden of lung cancer continues to increase, new agents are being developed for more effective treatment and palliation of symptoms. Lung cancer has been treated with a wide range of modalities, including surgery, radiotherapy, and chemotherapy, as first and second lines of treatment. However, many patients experience relapse after cytotoxic chemotherapy, whereas they are still in a competent physical status.

Gefitinib (ZD1839, Iressa) is an epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor. Although the Iressa Non–Small Cell Lung Carcinoma (NSCLC) Trial Assessing Combination Therapy study (2, 3) showed no benefit of adding gefitinib to cytotoxic chemotherapy, two phase II monotherapy trials (Iressa Dose Evaluation in Advanced Lung Cancer; refs. 4, 5) showed response rates of 10% to 20% with single-agent gefitinib in relapsed refractory NSCLC. Considering the increasing demand for third or fourth lines of treatment and the results of previous trials, a recent American Society of Clinical Oncology guideline (6) adopted gefitinib as a third line of treatment.

EGFR mediates cancer cell growth, proliferation, invasion, and metastasis, and inhibits apoptosis (7). Studies showed that gefitinib targets the ATP cleft within the tyrosine kinase domain of EGFR that is triggered by the binding of EGF. The phosphorylation of EGFR through tyrosine kinase domains inhibits the growth of human tumors (8). These results indicate that gefitinib has potential for treating lung cancers that are dependent on the activation of the EGFR pathway.

Despite a low response rate, the very rapid and often complete response to gefitinib in a subgroup of patients with NSCLC led us to search for predictors of the response. Although female gender, the presence of adenocarcinoma, and no smoking history might be markers of a favorable response, mutations of the *EGFR* gene are reported to show high positive predictive value for a response (9, 10).

In South Korea, gefitinib was used to treat 961 patients with NSCLC in the Expanded Access Program between December 2002 and July 2003. At our institution, we enrolled 70 patients in this program; after gefitinib became available commercially, 28 more patients were treated with this drug. This is a retrospective review of 98 NSCLC patients who were treated with gefitinib in a single Korean institution.

PATIENTS AND METHODS

Patients. Seventy patients were enrolled in the Expanded Access Program after they showed evidence of relapse after at least one course of cytotoxic chemotherapy. After it was approved by the Korean Food and Drug Administration,

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a commercial form of gefitinib was given to 28 more patients. Therefore, 98 patients were included in this analysis. The characteristics of the subjects are summarized in Table 1. Eleven patients were labeled as having non–small cell lung cancer because their diagnoses were made by cytologic examination and the histologic type could not be differentiated further.

For most of the patients, gefitinib was the second ($n = 44$), third ($n = 45$), or fourth ($n = 5$) line of treatment for refractory relapse after previous cytotoxic chemotherapy, whereas gefitinib was given as the first-line treatment for four patients. The cytotoxic chemotherapy consisted of platinum-based combination regimens with paclitaxel, docetaxel, gemcitabine, etoposide, or vinorelbine. This study was carried out in accordance with the institutional review board of our institution, and written informed consent was obtained from all of the patients for use of gefitinib and potential studies with their tumor or blood specimen.

Treatment and Response Evaluation. Gefitinib was given at a dose of 250 mg once a day for between 5 and 952 days. To evaluate the therapeutic response and adverse reactions, chest radiographs, computed tomography (CT) scans, complete blood cell counts, and blood chemistries were monitored. We classified the therapeutic results as complete or partial remission and stable or progressive disease according to the response evaluation criteria for solid tumors (11).

The response could be evaluated in 80 patients who maintained treatment for more than 4 weeks. By the time the patients began to take gefitinib, 16 patients were in poor condition and could not maintain treatment for more than 4 weeks. Adverse events were graded using the Common Terminology Criteria for Adverse Events, version 3.0 (December 2003, http://ctep.cancer.gov). One patient discontinued gefitinib because of skin eruptions (grade 3) and two patients because of interstitial pneumonia (grades 1 and 5).

Table 1 Characteristics of the subjects

Number of patients	98
Age (mean ± SD, range, y)	62.7 ± 9.8 (30-80)
Sex (male/female)	76/22
Smoking history	Missing = 15
Never-smoked	17
Ex-smoker (quit >1 y before diagnosis)	12
Current smoker	54
Histology	
ADC	41
BAC	6
SQC	40
NSCLC	11
ADC + BAC vs. non-ADC	47 vs. 51
Gefitinib treatment	
First-line therapy	4
Second-line therapy	44
Third-line therapy	45
Fourth-line therapy	5
Response to gefitinib	Evaluable patients = 80
PR	20 (25%)
SD	18 (22.5%)
PD	42 (52.5%)

Abbreviations: ADC, adenocarcinoma; BAC, bronchiolo-alveolar cell carcinoma; SQC, squamous cell carcinoma; PR, partial remission; SD, stable disease; PD, progressive disease.

Immunohistochemical Staining for Epidermal Growth Factor Receptor Protein. The cancerous tissues from 30 patients were stained immunohistochemically for the EGFR. All the steps in the staining procedure were done using the Microprobe System (Fisher Scientific, Pittsburgh, PA), taking advantage of capillary gap action (12). The tissue section was exposed to primary antibody for EGFR (Zymed, 31G7, San Francisco, CA) for 20 minutes after blocking endogenous peroxidase activity with Autoblocker (Research Genetics, Huntsville, AL) for 5 minutes. Antigen/antibody complexes were detected with a goat anti-mouse antibody (Sigma, St. Louis, MO) for 10 minutes followed by streptavidin-horseradish peroxidase (DAKO, Glostrup, Denmark) for 10 minutes. The chromogen reaction consisted of liquid 3,3′-diaminobenzidine (DAKO) for 10 minutes followed by a 30-second application of hematoxylin. Then, the sections were mounted in Faramount (DAKO).

Negative controls consisted of staining with primary antibody diluent (Research Genetics) only. All the slides were examined using standard light microscopy and were scored independently by two observers in quartiles as staining intensity from grades 1 to 4.

Sequence Analysis of the *EGFR* Gene. Twenty-seven tumor specimens (8 responders and 19 nonresponders) were available for the sequence analysis of exons 18, 19, and 21 of the *EGFR* gene. Tumor DNA and matched normal DNA were extracted from a paraffin-embedded tissue block and peripheral blood buffy coat. After deparaffinization in xylene, DNA was extracted using a Gene All Tissue DNA Purification kit (General Biosystem, Seoul, South Korea).

For the PCR, three sets of previously reported primers (10) were used. Using a Perkin-Elmer 2400 thermal cycler (Perkin-Elmer Corp., Norwalk, CT), PCR was carried out with an initial 5-minute denaturation at 94°C, followed by 40 cycles of 30 seconds at 94°C (denaturation), 30 seconds at 58°C (annealing), and 1 minute at 72°C (extension), and then by 10 minutes at 72°C and a final cooling to 4°C. For one PCR reaction, 100 ng of template DNA were mixed with 1.5 μL of each primer at a concentration of 10 pmol, 2 units of Taq DNA polymerase (Super Bio, Seoul, South Korea), 0.2 mmol/L deoxynucleotide triphosphate (Super Bio), 3 mmol/L $MgCl_2$, 100 mmol/L KCl, and 17 μL sterilized distilled water. Pure water was used to replace the template (DNA) as a negative control for every PCR experiment. The PCR products were confirmed by 2% agarose gel electrophoresis.

For sequencing, the PCR products were purified using a Gene All PCR Purification kit (General Biosystem). Sequencing was done using an ABI Prism 3100 Genetic Analyzer (PE Biosystems, Foster City, CA), according to the protocol of the manufacturer; the reagent mixtures contained 1 μL of 1.6 pmol forward primer, 1 μL Big Dye Terminator, 3.5 μL Thermo Sequence reagent, and 1.5 μL sterilized distilled water. The reaction tubes were placed in the thermal cycler, and amplification was started with an initial heating at 96°C for 1 minute, followed by 25 cycles of 94°C for 10 seconds, 50°C for 30 seconds, and 60°C for 4 minutes, with a final cooling to 4°C. After amplification and ethanol precipitation, the products were sequenced. Sequences with deletion or mutation were verified with both forward and reverse sequencing analyses.

Statistical Analysis. Statistical analyses were done using SPSS for Windows version 12.0 (SPSS, Inc., Chicago, IL). Descriptive statistics, frequency tables, χ^2 tests, and univariate and multivariate binary logistic regression analyses were used. Predictors of response were investigated, including age, nature of prior therapies and time elapsed since prior therapy before gefitinib, gender, histology, and smoking history.

Survival time and time to progression were recorded as days from the beginning of gefitinib treatment. Kaplan-Meier method was used to calculate survival and time to progression. Univariate analyses of survival and time to progression according to predictors were done using a log-rank test.

RESULTS

Clinical Response to Gefitinib Treatment. After receiving gefitinib for at least 4 weeks, 42 patients showed progression of their disease, 18 patients remained stable, and 20 patients obtained partial remission. The remission rate was 25% (20/80) and the disease control rate (partial remission + stable disease) was 47.5% (Table 1).

In 20 responders, improved radiologic findings and tumor-related symptoms were noted within 4 weeks. Figure 1 shows an index case that displayed rapid resolution. Within 7 days of gefitinib administration, a rapid reduction of bronchorrhea and a resolution of pulmonary infiltrate were noted, and the subject has been maintained on gefitinib for more than 520 days.

Adverse Events with Gefitinib Treatment. Adverse events including skin rash, acne, anorexia, malaise, and diarrhea were noted. Skin rash was observed in the majority of patients. Grades 1 to 2 anorexia, malaise, and diarrhea were observed in 52.8%, 38.9%, and 19.4%, respectively (Table 2).

Two patients developed possible gefitinib-induced interstitial pneumonia. A 71-year-old male with squamous cell carcinoma noted progressive dyspnea after taking gefitinib for 2 weeks and 2 additional weeks without gefitinib. On admission, faint bilateral interstitial pulmonary infiltrates were noted. No further study was possible as the patient died of circulatory collapse 2 days after admission. The causality and response to gefitinib could not be evaluated independently with this patient.

A 69-year-old male with squamous cell carcinoma had been taking gefitinib for 56 days when he returned to the clinic for response evaluation. The chest radiography and CT scan (Fig. 2*A*) showed interstitial infiltrates and ground-glass attenuations. Therefore, we discontinued gefitinib and started empirical therapy with prednisolone and clarithromycin. After 2 weeks, the chest CT (Fig. 2*B*) showed resolution of the interstitial infiltrates and the interstitial pneumonia did not recur. The response to gefitinib was classified as stable disease.

Predictors of the Response to Gefitinib. The response to gefitinib was positively correlated with adenocarcinoma, never having smoked, female gender, and age. Response rate was significantly higher for adenocarcinoma (41.0%) versus non-adenocarcinoma (9.8%, $P = 0.001$), in those who never smoked (58.8%) versus smokers (15.9%, $P < 0.001$), in age ≤65 (35.0%) versus age > 65 years (15.0%, $P = 0.039$), and in females (42.1%) versus males (19.7%, $P = 0.049$). The degree of improvement of tumor-related symptoms was also significantly positively correlated with the response (Table 3).

The response was not related to the degree of skin rash, other adverse events, or the number and previous chemotherapy regimens used before gefitinib. However, the time elapsed since prior therapy before gefitinib was significantly shorter in responders (84.7 ± 86.1 days) than in nonresponders (162.6 ± 86.1 days, $P = 0.012$). All the cancerous tissues examined showed positive EGFR staining. Grades 1 to 2 staining intensity was noted in 12 patients, and grades 3 to 4 in 18 patients. There was no significant correlation between response rate and EGFR staining intensity (Table 3).

In a multivariate logistic analysis using the variables that were significant in the univariate analyses, the independent predictors of response were smoking history [odds ratio, 0.21; 95% confidence interval (95% CI), 0.06-0.72; $P = 0.01$] and

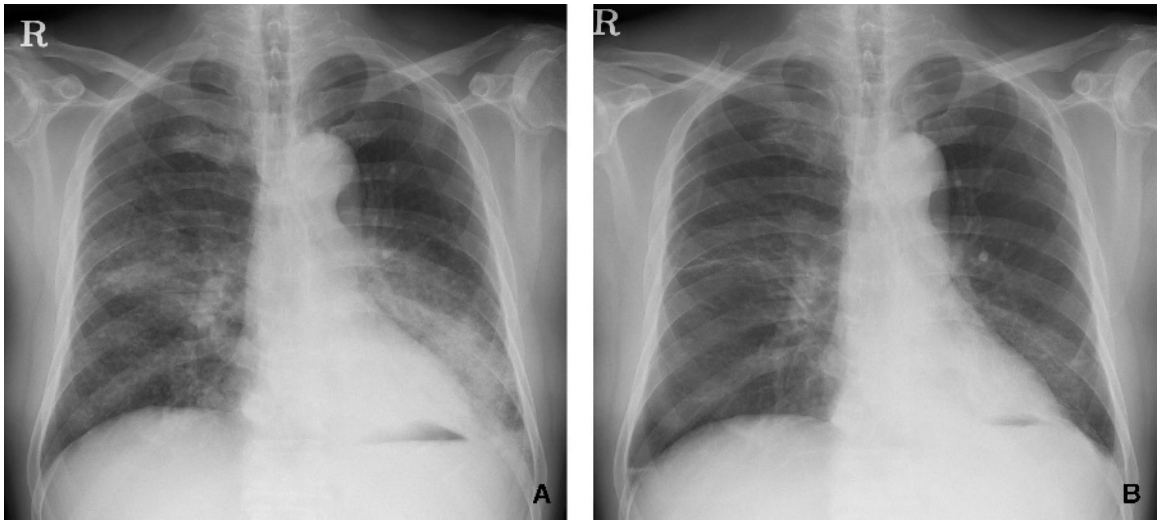

Fig. 1 Chest radiography of a 73-year-old man who showed a rapid response to gefitinib. After the diagnosis of bronchiolo-alveolar cell carcinoma, the patient received two cycles of cytotoxic chemotherapy, but progression was noted (*A*). Within a week of gefitinib treatment, rapid and marked resolution of infiltrates and bronchorrhea were observed (*B*).

Table 2 Adverse events and symptoms according to the Common Terminology Criteria for Adverse Events version 3.0

	Common Terminology Criteria grade		
	0	1-2	3-4 or 5*
Adverse events	% (*n*)	% (*n*)	% (*n*)
Skin rash	2.7% (2)	94.6% (70)	2.7% (2)
Acne	47.3% (35)	51.4% (38)	1.4% (1)
Anorexia	44.4% (32)	52.8% (38)	2.8% (2)
Malaise	59.7% (43)	38.9% (28)	1.4% (1)
Diarrhea	80.6% (58)	19.4% (14)	
Interstitial pneumonia	97.2% (70)	1.4% (1)	1.4% (1)*

*Grade 5 (death).

adenocarcinoma (odds ratio, 4.30; 95% CI, 1.19-15.49; $P = 0.03$). Therefore, we classified the subjects according to smoking history and histologic type. The response rate of the subgroup with non-adenocarcinoma and smoking history was 7.9% (3/38), that of the subgroup with either adenocarcinoma or never-smoked was 28.6% (8/28), and that of the subgroup with adenocarcinoma and no smoking history was 64.3% (9/14, $P < 0.001$).

Sequencing Results and Correlation with the Response to Gefitinib. The *EGFR* gene was sequenced in 27 cases (8 partial remission, 19 stable disease + progressive disease). No mutation was found in the 19 nonresponders, whereas six of eight responders had mutations in the *EGFR* gene. Homozygous ($n = 1$) or heterozygous ($n = 4$) deletions of exon 19 were found in five responders. The deletions involved 15 or 18 bases between nucleotides 2,235 and 2,253, spanning codons 746 to 751 within the kinase domain of EGFR. DNA with a homozygous deletion was obtained from metastatic brain tumor tissue, and the samples with heterozygous deletions were from primary lung cancer tissue. The point mutation in exon 21 found in one responder was also heterozygous and involved an L858R amino acid substitution. The regions at which deletions and point mutations occurred were similar to those previously reported (refs. 9, 10; Table 4 and Fig. 3). None of the matched DNA from peripheral blood leukocytes showed mutations in the *EGFR* gene.

A response to gefitinib was found in all the patients with a mutation (100% response rate), whereas the response rate was 9.5% (2/21) in the patients without a mutation ($P < 0.001$). Although never-smoked and adenocarcinoma were independent predictors of the response, they were not as specific as the genetic profile because 9.5% (2/21) of smokers and 6.7% (1/15) of those with non-adenocarcinoma had mutations of the *EGFR* gene. As there were only 27 cases with tumor DNA, the sequencing results were not entered in the multivariate logistic analysis.

Survival and Time to Progression Analyses. The median survival time was 173 days (95% CI, 116-230 days). Median survival was significantly shorter in patients with progressive disease (130 days; 95% CI, 116-144 days) or stable disease (141 days; 95% CI, 52-230 days) compared with those who showed partial remission (618 days; 95% CI, 554-682 days, log-rank $P < 0.001$, Fig. 4*A*).

Twenty-seven patients showed progression of disease among patients who showed stable disease (17/18) or partial remission (10/20), and their median time to progression was

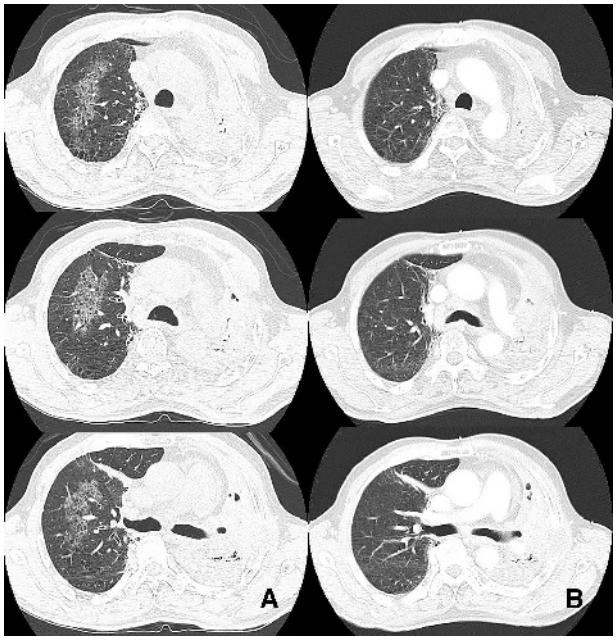

Fig. 2 Interstitial pneumonia in a patient receiving gefitinib. The chest CT scan (*A*) showed ground-glass attenuations. Gefitinib was discontinued and empirical therapy with prednisolone and clarithromycin was started. After 2 weeks, the chest CT scan (*B*) showed resolution of the interstitial pneumonia.

Table 3 Comparison of the response rate by possible predictor of response (χ^2 test)

	Number of responders/ total number	Response rate (%)	Significance
Age (y)			
≤65	14/40	35.0	
>65	6/40	15.0	$P = 0.039$
Sex			
Male	12/61	19.7	
Female	8/19	42.1	$P = 0.049$
Smoking history			
Smoker	10/63	15.9	
Never-smoked	10/17	58.8	$P < 0.001$
Histology			
Non-adenocarcinoma	4/41	9.8	
Adenocarcinoma	16/39	41.0	$P = 0.001$
EGFR staining intensity			
1-2	1/12	8.3	
3-4	5/18	27.8	$P = 0.192$
Prior chemotherapy			
Taxanes	3/20	15.0	
Gemcitabine	4/13	30.8	
Taxanes and gemcitabine	10/41	24.4	$P = 0.546$
Courses of prior chemotherapy			
≤1	10/36	27.8	
≥2	10/44	22.7	$P = 0.604$
Acneiform skin eruption			
=0	6/32	18.8	
≥1	13/37	35.1	$P = 0.129$
Symptomatic improvement			
No	1/48	2.1	
Yes	17/19	89.5	$P < 0.001$

Abbreviations: SQC, squamous cell carcinoma; ADC, adenocarcinoma; BAC, bronchiolo-alveolar cell carcinoma.

Table 4 Characteristics of responders to gefitinib and mutations in the *EGFR* gene

Case nos.	Histology	Tissue	Sex/Age	Smoking	Exon 18	Exon 19	Exon 21
1	SQC	Metastatic brain tumor	F/54	Never	Wild-type	Del (Hm) 2,235-2,249	Wild-type
2	ADC	Lung tumor	F/48	Never	Wild-type	Del (Ht) 2,235-2,249	Wild-type
3	BAC	Lung tumor	M/74	Yes	Wild-type	Del (Ht) 2,236-2,250	Wild-type
4	BAC	Lung tumor	M/67	Yes	Wild-type	Del (Ht) 2,235-2,249	Wild-type
5	ADC	Lung tumor	M/78	Never	Wild-type	Del (Ht) 2,236-2,253, substitution of T for C at 2,236	Wild-type
6	ADC	Lung tumor	F/54	Never	Wild-type	Wild-type	Substitution of G for T at 2,573 (Ht)
7	SQC	Lung tumor	M/80	Yes	Wild-type	Wild-type	Wild-type
8	ADC	Lung tumor	M/53	Yes	Wild-type	Wild-type	Wild-type

Abbreviations: Hm, homozygous; Ht, heterozygous.

258 days (95% CI, 168-348 days). Median time to progression was also significantly shorter in patients with stable disease (75 days; 95% CI, 0-156 days) compared with those who showed partial remission (451 days; 95% CI, 318-584 days, log-rank $P < 0.001$, Fig. 4*B*).

Survival and time to progression according to predictors of response were summarized in Table 5. The smoking history, histologic type, and mutation of *EGFR* gene were significantly correlated with survival.

DISCUSSION

Cytotoxic chemotherapy improves the survival of unresectable NSCLC (6), and thus there is increasing demand for more effective chemotherapeutic agents for NSCLC. The currently recommended platinum-based first line combination regimens use one of paclitaxel, docetaxel, gemcitabine, or vinorelbine. However, no combination regimen is superior to the others (13).

Patients with NSCLC respond to chemotherapeutic agents in different ways. We observe patients who respond to one regimen but not to the others. As no reliable chemosensitivity tests are available, we can only try two or more cycles until we can determine the response to a regimen. If we could predict the response to drugs before we use them, we should be able to offer more effective treatment whereas the patients are still in reasonable physical health.

Studies examining the status of target genes or target molecules of cytotoxic chemotherapeutic drugs are ongoing. For example, platinum compounds act by making DNA adducts in tumor cells. If the DNA-repair enzyme activity is high in tumor cells, platinum-based chemotherapy may be less effective. The mRNA of excision repair cross-complementation group 1 (ERCC1) expression was reported to be a predictor of survival in patients treated with platinum-based chemotherapy (14). A polymorphism of the *ERCC1* gene, which alters the activity of the enzyme, was also reported to predict survival in patients with NSCLC who were treated with platinum-based chemotherapy (15).

Recently, the mRNA of the M1 subunit of ribonucleotide reductase, a housekeeping enzyme that supplies DNA substrates, was also reported to predict survival in gemcitabine-treated patients (16). As microtubules are targets of taxanes, mutations of tubulin genes are also possible predictors of the response to taxanes (17). However, more data are needed before this can be used clinically because of the weak correlations with the response to chemotherapy.

Conversely, predicting the response would be easier for targeted therapies. In breast cancer, expression of HER2 protein and *HER2* gene amplifications predict the response to HER2 monoclonal antibody (trastuzumab; ref. 18). However, the expression of EGFR is not correlated with the response to

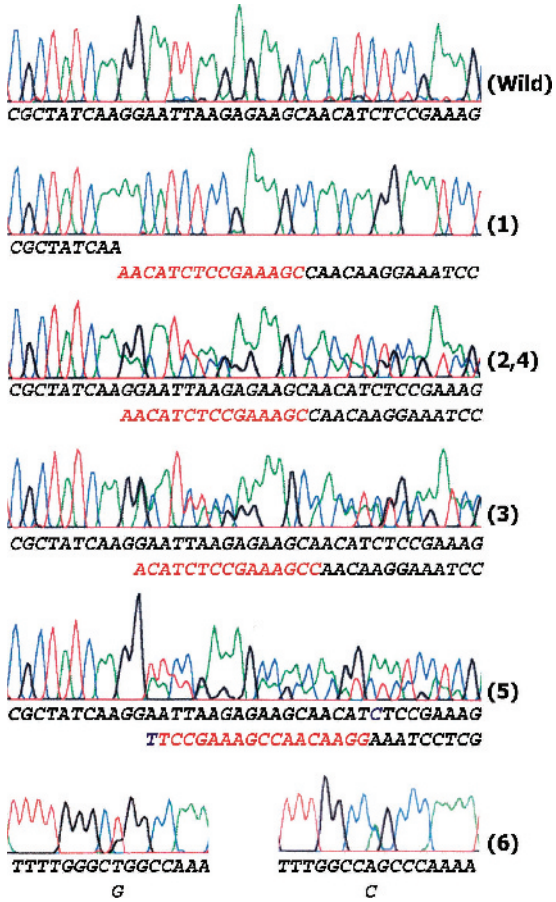

Fig. 3 Mutations in the *EGFR* genes of gefitinib-responsive patients. Note the homozygous (case 1) or heterozygous (cases 2-5) deletion of exon 19 of the *EGFR* gene. Forward and reverse sequencing shows the point mutation in exon 21, which is heterozygous (case 6). The case numbers represent the patient numbers in Table 4, and "Wild" is a normal DNA sequence of exon 19 from a nonresponder.

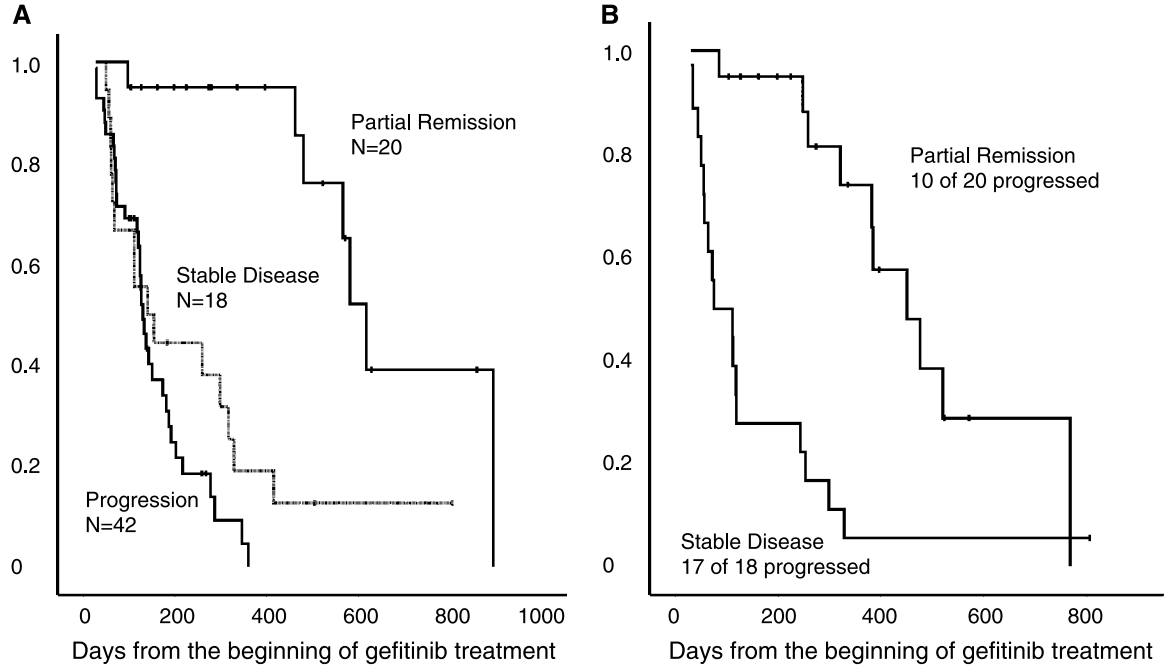

Fig. 4 Kaplan-Meier estimates of survival (*A*) and time to progression (*B*) of patients treated with gefitinib according to response to gefitinib.

gefitinib. Gefitinib has anticancer activity via the dose-dependent inhibition of EGFR autophosphorylation in tumor cell lines (19). Nevertheless, tumor cell growth inhibition by gefitinib is not strongly influenced by the level of EGFR expression (20). In our study, the EGFR staining intensity did not differ with the therapeutic response to gefitinib in 30 patients.

Although the expression of EGFR protein was not a reliable predictor of the response to gefitinib, the pathologic type adenocarcinoma, female gender, and lifetime non-tobacco use were reported to be associated with a good response rate or survival (21–24). Our data concur with those previous results (i.e., those three variables have high predictability). Nevertheless, in the multivariate analysis, female gender lost its significance, and the independent predictors of the response were adenocarcinoma and never-smoked. In view of the higher probability of a response in patients with adenocarcinoma who never smoked, an adenocarcinoma that developed in nonsmoking patients is more likely to have different characteristics in the *EGFR* gene or its expression.

Two recent studies (9,10) found mutations in the *EGFR* gene only in responders to gefitinib. Most of the responders had a deletion in exon 19 or point mutations in exon 18 or 21 of the *EGFR* gene, whereas none of the nonresponders had these mutations. Moreover, these mutations of *EGFR* doubled or tripled the EGF signal and prolonged the activation compared with the wild-type receptor. The mutations are thought to be correlated with gefitinib sensitivity because the mutant receptors on tumor cells are significantly more sensitive to gefitinib and play a significant role.

Table 5 Survival and time to progression according to predictors of response

	Definition	Median survival days (95% CI)	*P*	Median time to progression days (95% CI)	*P*
Response	SD or PD	133 (114-152)		75 (0-156)	
	PR	618 (554-682)	< 0.001	451 (318-584)	< 0.001
Age (y)	≤65	173 (97-249)		382 (168-596)	
	>65	187 (107-267)	= 0.273	243 (54-432)	= 0.639
Sex	Male	141 (112-170)		299 (9-589)	
	Female	287 (205-369)	= 0.114	258 (245-271)	= 0.872
Smoking	Smoker	141 (112-170)		243 (29-457)	
	Never-smoked	567 (17-1,117)	= 0.009	382 (91-673)	= 0.176
Histologic type	Non-ADC	133 (108-158)		111 (38-184)	
	ADC	299 (156-442)	= 0.009	385 (211-559)	= 0.002
EGFR expression	Grade 1-2	111 (50-172)		111 (27-195)	
	Grade 3-4	126 (33-219)	= 0.169	258 (0-539)	= 0.314
EGFR mutation	Wild-type sequence	143 (90-196)		85 (17-153)	
	Mutation	567 (not calculated)	= 0.008	382 (187-577)	= 0.003

Abrreviations: CI, confidence interval; SD, stable disease; PD, progressive disease; PR, partial remission; ADC, adenocarcinoma.

A subsequent study (25) showed that these EGFR mutants selectively activate the Akt and signal transducers and activators of transcription signaling pathways, which promote cell survival. NSCLC cells expressing EGFR mutants underwent extensive apoptosis after knockdown of the mutant EGFR. Therefore, it is possible that inhibiting the mutant EGFR causes tumor cell apoptosis and a rapid therapeutic effect.

In this study, we found similar deletions in exon 19 and mutations in exon 21, which are the coding sequences for the amino acids in the tyrosine kinase domains of EGFR. The matched normal DNA showed no mutations, and thus EGFR mutations must be somatic in origin. The heterozygous nature of somatic mutations implies that they exert a dominant oncogenic effect in tumor cells. However, neither the previous studies (9, 10) nor ours used a laser capture technique to extract tumor DNA specifically; normal lung DNA might have been included. Therefore, the gefitinib-responsive mutations may be homozygous, as seen in DNA from a metastatic brain tumor in our study and a case from Paez et al. (9).

The incidence and severity of adverse events were similar to those in previous reports (4, 5). We experienced two cases (2.8%) with interstitial pneumonia possibly associated with gefitinib. The reported incidence of interstitial pneumonia is 1% worldwide and about 0.3% and 2% in the United States and Japan, respectively. After interstitial pneumonia develops, the mortality is about 30% (26). Although the mechanism and risk factors of interstitial pneumonia are not yet understood, underlying pulmonary fibrosis was reported as a risk factor (27). One should look for this possible adverse reaction with serial X-rays at follow-up, especially during the first few months of treatment (27–29).

In the present report, we have summarized our findings on the effects of gefitinib in 98 Korean patients with NSCLC. We observed responses and adverse events similar to those reported previously, and the independent predictors of a response were never-smoked and adenocarcinoma. We also observed mutations of the *EGFR* gene as a highly predictive marker of the response to gefitinib. The predictors of response showed significant correlations with survival and time to progression.

In conclusion, the genetic profile may replace those variables as an independent predictor of response because about 10% of smokers and of those with non-adenocarcinoma also showed mutations of the *EGFR* gene. The high positive-predictive value of mutations suggests the possibility of tailored therapy for patients with NSCLC, even as a first or second line of treatment.

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