Low-Dose Gefitinib Treatment for Patients with Advanced Non-small Cell Lung Cancer Harboring Sensitive Epidermal Growth Factor Receptor Mutations

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Introduction: Although standard schedule of gefitinib was the administration of 250 mg tablet every day, many patients need dose reduction because of toxicities. However, the efficacy of such low-dose gefitinib for patients with epidermal growth factor receptor-mutated non-small cell lung cancer has rarely been evaluated.

Methods: A post hoc comparison of the efficacy (response rate and survival) in patients treated with gefitinib with or without any dose reduction in NEJ002 study was performed.

Results: Among 114 patients treated with first-line gefitinib in NEJ002, 61 (54%) continued gefitinib without any dose reduction until their diseases progressed, and 53 (46%) reduced their dose of gefitinib because of some toxicities. There was no significant difference of patient characteristics between the two groups. The progression-free survival of low-dose group tended to be better than that of standard-dose group (median progression-free survival, 11.8 versus 9.9 months; $p = 0.144$), and the overall survival of low-dose group was also better than that of standard-dose group (median survival time, 32.7 versus 25.3 months; $p = 0.049$).

Conclusions: The results suggest that low-dose gefitinib may be clinically not inferior to standard-dose gefitinib for non-small cell lung cancer with sensitive epidermal growth factor receptor mutations. Prospective study of low-dose gefitinib is warranted especially for frail patients who need less toxic treatment.

Key Words: Gefitinib, EGFR mutation, Low-dose, Post hoc analysis.

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Cytotoxic chemotherapy such as carboplatin (CBDCA) plus paclitaxel (PTX) had been the standard first-line treatment for advanced non-small cell lung cancer (NSCLC) patients for a long time; however, its efficacy had already reached a plateau in early 2000s, and better treatment strategies have been eagerly anticipated.[1,2] Gefitinib is the first molecular-targeted agent for NSCLC and is classified as a tyrosine kinase inhibitor of the epidermal growth factor receptor (EGFR-TKI).[3] Although gefitinib was initially approved for the entire NSCLC population, pivotal studies published in 2004 had revealed that the presence of somatic mutations in the kinase domain of EGFR strongly correlates with an increased responsiveness to EGFR-TKI.[4,5] Biomarker analysis performed in Iressa Pan-Asia Study, in which efficacy of gefitinib and CBDCA/PTX was compared as the first-line treatment for NSCLC patients with favorable clinical characteristics including adenocarcinoma and nonsmoking history, showed a significant superiority of gefitinib in progression-free survival (PFS) in the subset analysis for NSCLC with mutated EGFR.[6] Recently, we prospectively demonstrated in NEJ002 phase 3 study that the first-line gefitinib exhibited a significantly longer PFS than CBDCA/PTX in patients with advanced NSCLC with mutated EGFR.[7] According to these results, EGFR-TKI has become one of the

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standard treatments for advanced NSCLC with mutated EGFR.

A standard dosage of gefitinib is 250 mg, which is administered every day. Nevertheless, not a few patients need a dose reduction of gefitinib due to toxicities including rash, diarrhea, or liver dysfunction. Because the tablet of gefitinib cannot be divided in half, the dose reduction is usually achieved by changing the interval of taking the tablet from every day to every 2 days. However, clinical evidence of such reduced dose of gefitinib is scanty. According to some preclinical data, lung cancer cell harboring sensitive EGFR mutation are much more sensitive to EGFR-TKI than those with wild-type EGFR.[4] Therefore, we hypothesized that selected patients on the basis of EGFR mutations might sufficiently and safely benefit from such "low-dose" gefitinib. The aim of this post hoc analysis from NEJ002 is to examine the efficacy of low-dose gefitinib compared with that of standard-dose gefitinib in EGFR-mutated NSCLC patients.

METHODS

Patient Population

We retrospectively analyzed the 114 patients treated with gefitinib in NEJ002 study, which is a multicenter, randomized, phase 3 trial that compared gefitinib with CBDCA/TXL as the first-line treatment for advanced NSCLC harboring sensitive EGFR mutations. Eligibility criteria of NEJ002 included the presence of advanced NSCLC harboring sensitive EGFR mutations without the resistant EGFR mutation T790M examined by PNA-LNA polymerase chain reaction clamp method,[8] no history of chemotherapy, an age of 75 years or younger, performance status 0 to 1, appropriate organ functions, and written informed consent.

Treatment with Gefitinib

All the patients initially received 250 mg of gefitinib everyday according to the protocol of NEJ002. In NEJ002, a temporary cessation of the drug administration was recommended by the protocol when an intolerable toxicity such as grade 3 or worse adverse event was observed during the treatment with the standard dose, and a dose reduction of gefitinib by changing the everyday schedule to every 2 days schedule was permitted when grade 2 toxicity was observed.

In this analysis, we categorized patients into two groups according to their treatment status as follows: standard-dose group, in which gefitinib was administered without any dose reductions until disease progression was observed, and low-dose group, in which gefitinib was administered with a reduced dose at least once during the treatment period before disease progression (Figure 1).

Clinical Assessments

According to the protocol of NEJ002, the assessment of antitumor response to gefitinib was performed by computed tomography every 2 months until disease progression was observed. Unidirectional measurements were adopted on the basis of the Response Evaluation Criteria in Solid Tumor (RECIST, version 1.0).[9] The PFS was defined as the period from the date of randomization to the date when disease progression was first observed or death occurred. The response and PFS were determined by an external review of the computed tomography films by experts who were not aware of the treatment assignment. Overall survival (OS) was defined as the period from the date of randomization to the date of death.

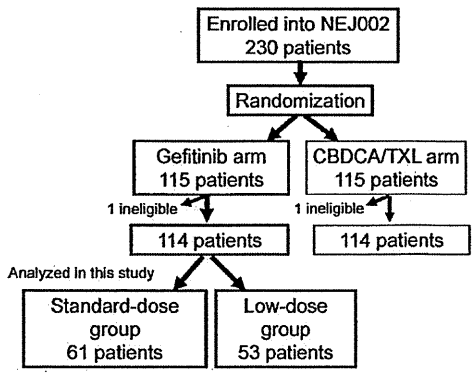

FIGURE 1. Flowchart of the patients analyzed in this study.

Kaplan-Meier survival curves were drawn for PFS and OS, and differences between the groups were compared by log-rank test. The difference in response rate was compared by Fisher's exact test. Each analysis was two-sided, with a 5% significance level and a 95% confidence interval, and was performed using SAS for Windows software (release 9.1, SAS Institute, Cary, NC).

RESULTS

Treatment

The demographics of patients in each group are listed in Table 1. Around half of the patients in NEJ002 were categorized as low-dose group. There was no significant difference in each clinical factor and the type of EGFR mutation between the two groups. Standard-dose group received 250 mg gefitinib for 261 days (median) (Table 2). Nine patients temporarily suspended their treatment (median, 6 days; range, 1–32 days) due to some toxicities but restarted the treatment with standard dose. Low-dose group received 250 mg gefitinib every day for 74 days (median) and then every 2 days for 125 days (median). Before restarting the treatment using a reduced dose, 38 patients needed a break in their treatment (median, 19 days; range, 2–79 days) to recover from adverse events.

At the data cutoff point (early December 2009), 37 patients (61%) in the standard-dose group and 26 patients (49%) in the low-dose group had stopped the first-line gefitinib treatment due to disease progression, while 7 patients (11%) in the standard-dose group and 5 patients (9%) in the low-dose group had terminated the treatment because of treatment-related toxicities such as interstitial lung disease and liver dysfunction.

Efficacy

Low-dose group showed at least not-inferior efficacy (response and survival) compared with standard-dose group.

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TABLE 1. Patient Characteristics

	Standard Dose	Low Dose	*p*
No. of patients	61	53	
Sex			
Male	27	15	0.084
Female	34	38	
Mean age (range)	64 (43–75)	64 (47–75)	0.742
Mean body weight (range)	56.2 (41.1–81.6)	54.2 (34.7–93.0)	0.443
Smoking status			
Never smoker	37	38	0.876
Smoker	24	15	
Performance status			
0/1/2	28/33/0	26/26/1	0.824
Histology			
Adenocarcinoma	53	50	0.483
Others	8	3	
Clinical stage			
IIIB	8	7	0.805
IV	46	42	
Postoperative	7	4	
Type of EGFR mutation			
Exon 19 deletion	27	31	
L858R	27	22	
Others	7	0	

TABLE 2. Treatment Pattern with Gefitinib in Each Group

	Standard Dose	Low Dose
Given continuously	*n* = 61	*n* = 53
Mean (SD)	287 d (211)	160 d (197)
Median (range)	261 d (14–790)	74 d (19–1153)
Given intermittently		*n* = 53
Mean (SD)	—	205 d (200)
Median (range)	—	125 d (7–897)
Treatment break period	*n* = 9	*n* = 38
Mean (SD)	13 d (11)	23 d (18)
Median (range)	6 d (1–32)	19 d (2–79)

The response rate and disease control rate were 83% and 98% in the low-dose group and 66% and 82% in standard-dose group, respectively.

PFS for low-dose group tended to be superior to that of standard group, although a statistical significance was not detected. Median PFS and 1-year PFS rate were 11.8 months and 50% in low-dose group and 9.9 months and 36% in standard-dose group, respectively (Figure 2*A*). As some patients in low-dose group had switched to the low dose after a long-term treatment with standard-dose gefitinib, we additionally investigated the efficacy of more "refined" low-dose group (n = 25) who had been treated with gefitinib at standard dose during less than 60 days. The response rate, median PFS, and 1-year PFS rate of the group were 83%, 7.1 months, and 27%, respectively, which was not statistically different from those results of standard-dose group (Figure 2*B*). The OS was significantly longer in low-dose group than standard-dose group (median: 32.7 versus 25.3 months; $p = 0.049$) (Figure 2*C*, Table 3).

DISCUSSION

Recent phase 3 studies including NEJ002 have suggested that EGFR-TKIs are more effective than cytotoxic chemotherapy in the first-line treatment against advanced NSCLC with mutated EGFR.[6,7,10] However, many patients could not continuously receive standard dose of gefitinib because of some adverse effects. In fact, about half of the patients treated with gefitinib in NEJ002 required a dose reduction. Therefore, treatment strategy with less toxicity is required especially for patients with a poor condition or for elderly patients. In this report, we demonstrated that low-dose gefitinib may not be inferior to standard-dose gefitinib for NSCLC patients with EGFR mutations.

Previous reports of EGFR mutations had suggested that NSCLC cell with mutated EGFR was highly sensitive to EGFR-TKI than those without mutations.[5] Recently, Yeo et al.[11] also showed that both erlotinib and gefitinib suppressed the proliferation of EGFR-mutated NSCLC cell lines even at a very low concentration. Moreover, they reported a retrospective observation that patients treated with 25 mg of erlotinib which was equivalent to 250 mg of gefitinib where 5 out of examined 7 patients respond to the "low-dose" erlotinib and median PFS of those patients was 17 months. The current study employed a larger number of patients and supported their results that NSCLC patients with mutated EGFR received a similar level of efficacy from low-dose gefitinib as standard-dose gefitinib. Although low-dose gefitinib in this study are considered to be much less than 25 mg of erlotinib, twice longer half life in plasma and much higher tumor/plasma concentration ratio of gefitinib compared with erlotinib may favor gefitinib.[12–14]

There are some limitations in the current study. Because the study was a retrospective analysis, biases in patient characteristics or undetectable factors may exist and affected the results. From a pharmacokinetics point of view, as mean body weight tended to be lighter in the low-dose group, relatively higher drug concentration might be obtained in those patients even from the low-dose gefitinib. Considering that the OS for low-dose group was significantly longer than that for standard-dose group, the low-dose group might include more patients with slow-growing tumor than standard-dose group incidentally. More importantly, even for the patients in low-dose gefitinib group, the treatment was not initiated with low dose but with standard dose, thus the period with standard-dose gefitinib might affect the efficacy. Although the refined low-dose group still showed a similar efficacy to standard-dose group, its small sample size cannot draw a definite conclusion. To examine the efficacy and safety, and appropriate treatment schedule of low-dose gefitinib (e.g., initial standard dose followed by low dose versus thoroughly low dose), prospective comparative trials should be conducted.

In conclusion, our retrospective analysis suggests that low-dose gefitinib may be clinically equivalent to standard treatment with gefitinib for NSCLC with sensitive

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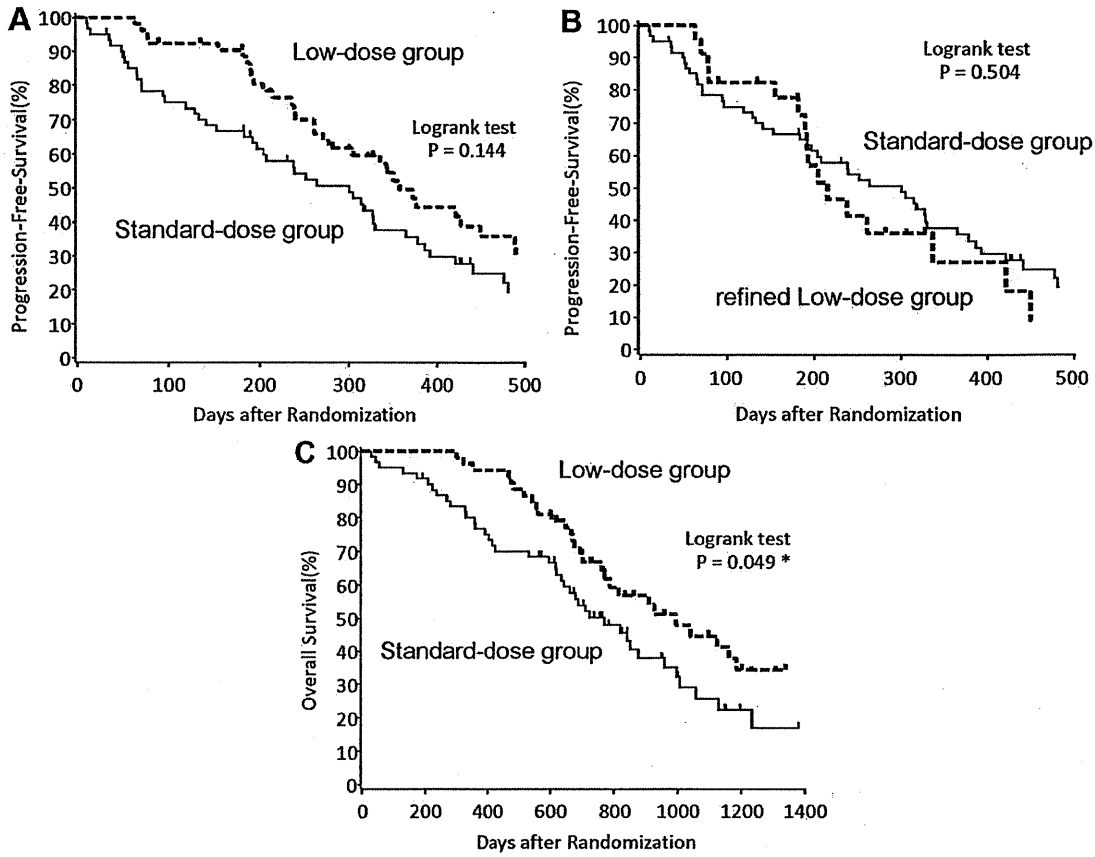

FIGURE 2. Progression-free survival curve of standard-dose group (solid line) and low-dose group (dotted line) with entire population (*A*) and another comparison of progression-free survival between standard-dose group and refined low-dose group (*B*). Overall survival curve of each group (*C*).

TABLE 3. Response and Survival

	Standard Dose	Low Dose	*p*
Overall response rate	66%	83%	0.005
95% CI	52–77	70–92	
Progression-free survival			
Median	9.9 mo	11.8 mo	0.144
1-yr PFS rate	36%	50%	
Overall survival			
Median	25.3 mo	32.7 mo	0.049
2-yr survival rate	50%	67%	

CI, confidence interval; PFS, progression-free survival.

EGFR mutations. Considering the merit of low-dose gefitinib in terms of risk-benefit balance, prospective studies using low-dose gefitinib is warranted targeting NSCLC patients with mutated EGFR, especially elderly or those with poor PS.

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Can Serum be Used for Analyzing the EGFR Mutation Status in Patients with Advanced Non-small Cell Lung Cancer?

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Background: Epidermal growth factor receptor (EGFR) mutations as prognostic or predictive marker in patients with non-small cell lung cancer (NSCLC) have been used widely. However, it may be difficult to get tumor tissue for analyzing the status of EGFR mutation status in large proportion of patients with advanced disease.

Patients and Methods: We obtained pairs of tumor and serum samples from 57 patients with advanced NSCLC, between March 2006 and January 2009. EGFR mutation status from tumor samples was analyzed by genomic polymerase chain reaction and direct sequence and EGFR mutation status from serum samples was determined by the peptide nucleic acid locked nucleic acid polymerase chain reaction clamp.

Results: EGFR mutations were detected in the serum samples of 11 patients and in the tumor samples of 12 patients. EGFR mutation status in the serum and tumor samples was consistent in 50 of the 57 pairs (87.7%). There was a high correlation between the mutations detected in serum sample and the mutations detected in the matched tumor sample (correlation index 0.62; $P < 0.001$). Twenty-two of 57 patients (38.5%) received EGFR-tyrosine kinase inhibitors as any line therapy. The response for EGFR-tyrosine kinase inhibitors was significantly associated with EGFR mutations in both tumor samples and serum samples ($P < 0.05$). There was no significant differences in overall survival according to the status of EGFR mutations in both serum and tumor samples ($P > 0.05$).

Conclusions: Serum sample might be alternatively used in the difficult time of getting tumor tissue for analyzing the status of EGFR mutation status in patients with advanced NSCLC.

Key Words: EGFR mutation, serum and tumor samples, NSCLC

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Lung cancer is a leading cause of cancer-related deaths worldwide,[1] with a 5-year survival of < 15%, because most patients are diagnosed with advanced stage disease.[2–5] Similarly, in Korea, lung cancer has a 15.5% 5-year survival.[6] Platinum-based combination chemotherapy has been considered as an initial standard therapy.[5,7,8] However, although patients with advanced non-small cell lung cancer (NSCLC) receiving platinum-based chemotherapy showed a modest but significant survival benefit compared with those with best supportive care (BSC) alone, the outcome of chemotherapy for NSCLC remains unsatisfactory. Representative molecularly targeted agents as a new approach for improving the outcomes in NSCLC are epidermal growth factor receptor tyrosine kinase inhibitors (EGFR-TKIs).[9–11] The small molecule EGFR-TKIs, gefitinib and erlotinib, have both demonstrated antitumor activity as a single agent in the treatment of patients with advanced NSCLC.[9–11]

Recently, IRESSA Pan-Asia Study trial for clinically selected NSCLC patients in East Asia demonstrated an outstanding survival benefit in patients with EGFR mutant tumors who received first-line EGFR-TKI therapy, which is superior to the outcomes of frontline cytotoxic chemotherapies, suggesting that the EGFR mutation is a useful predictive marker for selecting patients who may benefit from frontline EGFR-TKI treatment.[12] The Iressa NSCLC Trial Assessing Combination Treatment study did show a significant increased survival of EGFR mutation-positive patients treated with chemotherapy, irrespective of use of EGFR-TKI.[13] These findings are also in agreement with the molecular analysis of a phase III trial of erlotinib (TRIBUTE) in which EGFR mutations seemed to be a positive prognostic indicator irrespective of EGFR-TKI treatment.[14] These findings suggest EGFR mutations as prognostic or predictive marker in patients with NSCLCs have been meaningful.

Most EGFR mutations have been identified from surgical tissue. However, it is sometimes difficult to obtain tumor samples from patients with inoperable NSCLC and sufficient tumor DNA from nonsurgical tissue samples, for example, those derived from bronchoscopic biopsy. Even in prospectively conducted clinical trials, < 50% of the patients had tumors that were available for mutation analysis.[15] The same alterations have been observed in DNA from tumor samples and serum samples in patients with various types of tumors including NSCLC.[16,17] Several studies have demonstrated that EGFR mutations identical to those in the corresponding tumors can be detected in serum sample.[18] Serum sample can be obtained repeatedly and noninvasively from all NSCLC patients irrespective of patients' characteristics. In addition, the EGFR mutation test for serum sample should be rapid, sensitive, and inexpensive to perform. Some groups reported that the peptide nucleic acid locked nucleic acid (PNA-LNA) polymerase chain reaction (PCR) clamp method was a rapid, sensitive test for detecting sensitive EGFR mutations for NSCLC patients.[19–22] The detection of EGFR mutations in serum could be used effectively as a prognostic or

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predictive marker in advanced NSCLCs patients with or without EGFR-TKIs.

In this single institute, prospective study, we analyzed 57 patients with advanced NSCLC for EGFR mutations in axon 19 and 21 by using PNA-LNA method for serum samples and genomic PCR/direct sequence method for matched tumor tissue to ascertain the role of serum sample as an alternative tissue for EGFR mutation analysis. We also investigated EGFR mutation as a prognostic factor in advanced NSCLCs patients with or without EGFR-TKIs.

PATIENTS AND METHODS

Patients

Patients were required to have histologically proven stage IIIb to IV NSCLCs, an Eastern Cooperative Oncology Group performance status (ECOG PS) of 0 to 2 and no previous chemotherapy, between March 2006 and January 2009. As soon as patients were given a diagnosis of histologic NSCLC, serum samples from these patients were obtained. Only patients treated at the Korea University Medical Center were enrolled. The patients were prospectively observed for tumor responses and survival outcomes. Clinicopathologic data were recorded in electronic medical records and the registry of lung cancer at the Korean Lung and Breast Genomic Research Center at the Korea University, single institution. Clinicopathologic parameters recorded were as follows; sex, age at diagnosis, smoking history, ECOG PS, histologic type, stage, sites of metastasis, and the chemotherapy regimens. This study was approved by the Institutional Review Board at the Korea University Anam hospital. All patients signed informed consent to participate in this study and gave permission for the use of their serum and tumor tissue.

Tissue DNA Extraction and Amplification of EGFR Gene

DNA was extracted from five 10 μm-thick paraffin sections containing a representative portion of tumor tissue. DNA extraction from formalin-fixed paraffin-embedded tissue was carried out, using Gentra Puregene DNA purification kit (Qiagen, Hilden, Germany) as per the manufacturer's protocol. Fifty nanograms of DNA were amplified in a 20 μL reaction solution containing 10 μL of 2 X concentrated HotStarTaq Master Mix (Qiagen, Hilden, Germany), including PCR buffer, 3 mM $MgCl_2$, 400 μM each of dNTP, and 0.3 μM each of primer pairs (exon 18 F: 5′-CCA TGT CTG GCA CTG CTT T-3′, 18 R: 5′-CAG CTT GCA AGG ACT CTG A-3′, exon 19F: 5′-TGT GGC ACC ATC TCA CAA TTG-3′, 19R: 5′-GGA CCC CCA CAC AGC AA-3′, exon 20F: 5′- GGT CCA TGT GCC CCT CCT-3′, 20R: 5′- ATG GGA CAG GCA CTG ATT TGT-3′, exon 21F: 5′- GAC CCT GAA TTC GGA TGC a-3′, 21R: 5′- GCT AGT GGG AAG GCA GCC T-3′). Amplifications of EGFR (exon18 to 21) were performed using a 15-minute initial denaturation at 95°C; followed by 35 cycles of 30 seconds at 94°C, 30 seconds at 59°C, and 30 seconds at 72°C, and a 10-minute final extension at 72°C. PCR products were then 2% gel-purified with a QIAgen gel extraction kit (Qiagen, Hilden, Germany).

Direct Sequencing

DNA templates were processed for the DNA sequencing reaction using the ABI-PRISM BigDye Terminator version 3.1 (Applied Biosystems, Foster, CA) with both forward and reverse sequence-specific primers. Twenty nanograms of purified PCR products were used in a 10 μL sequencing reaction solution containing 1 μL of BigDye Terminator v3.1 and 0.1 μM of the same PCR primer. Sequencing reactions were performed using 25 cycles of 10 seconds at 96°C, 5 seconds at 50°C, and 4 minutes at 60°C, sequence data were generated with the ABI PRISM 3100 DNA Analyzer (Applied Biosystems, Foster, CA), and sequences were analyzed by Sequencing analysis 5.1.1 Software (Applied Biosystems, Foster, CA) to compare variations.

Serum Sample Collection and DNA Extraction

Blood samples were collected at the time of diagnosis in NSCLC. The volume of each blood sample was 6 mL. Serum was separated within 2 hours from sample collection and stored at −80°C until used. Serum DNA was extracted and purified by using a Qiamp Blood Kit (Qiagen, Hilden, Germany), with the following protocol modifications. One column was used repeatedly until the whole sample had been processed. The resulting DNA was eluted in 50 ul of sterile bidistilled buffer. The concentration and purity of the extracted DNA were determined by spectrophotometer. The extracted DNA was stored at −20°C until used.

PNA-LNA PCR Clamp

The PNA-LNA PCR clamp reaction was carried out using the Smart Cycler II (Cepheid, CA) as described by Hagiwara group.[19] PNA clamp primer suppressed the amplification of wild-type EGFR for mutant enriched condition. LNA probe significantly detected the mutant type EGFR. This reaction was briefly described as follows. All PCR reaction solutions (25 uL) were based on Pre Ex Taq (Perfect Real Time)

TABLE 1. Patients' Clinical and Disease Characteristics

Variables	No. of Patients (N=57)	% of Patients
Age (y), median (range)	64 (28-84)	
Younger than or equal to 65	31	54.4
Older than 65	26	45.6
Sex		
Male	35	61.4
Female	22	38.6
Smoking status		
Current or ever	32	56.1
Never	25	43.9
ECOG PS		
0-1	47	82.5
≤2	10	17.5
Histology		
Adenocarcinoma	40	70.2
Squamous cell carcinoma	17	29.8
Disease status		
3B	7	12.3
4	50	87.7
No. of metastatic sites		
0-1	26	45.6
≤2	31	54.4
First-line therapy		
Chemotherapy or EGFR-TKIs	39	68.4
No treatment	18	31.6
Use of EGFR-TKIs during treatment		
Yes	22	38.6
No	35	61.4
Line of EGFR-TKIs (N=22)		
First or second line	14	63.6
More than third line	8	36.4

ECOG PS indicates Eastern Cooperative Oncology Group performance status; EGFR-TKIs, epidermal growth factor receptor tyrosine kinase inhibitors.

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TABLE 2. Correlation of EGFR Mutations Between Serum DNA and Tumor DNA

	Tumor		
Correlate	EGFR Positive	EGFR Negative	Case No.
Serum			
EGFR Positive	8	3	11
EGFR Negative	4	42	46
Case number	12	45	57*

*Correlation index 0.62; $P<0.001$).
EGFR indicates epidermal growth factor receptor.

added to PCR primer (10 pmole), fluorogenic LNA probe (200 nM), and PNA clamp primer (5 uM). LNA probe was synthesized by IDT (Coralville, IA) and PNA was synthesized by PANAGENE (DaeJeon, Republic of Korea). PCR cycling was a 30-second hold at 95°C followed by 45 cycles of 95°C for 3 seconds and 56°C (exon19 and exon21) or 58°C (exon20) for 30 seconds.

Statistical Analysis

The χ^2 test or Fisher exact test was used to assess the association between EGFR mutation status and each of the clnicopathologic parameters. The relation between the EGFR mutations detected in serum and tumor samples was evaluated by the correlation analysis (P value and correlation index). A P value <0.05 was considered statistically significant. Overall survival (OS) according to EGFR mutation status in serum and/or tumor sample was estimated by the Kaplan-Meier method compared using the 2-sided log rank test. OS was defined as the time between the date of the diagnosis and the date of death from any cause. The 2-sided significance level was set at $P<0.05$. The Cox proportional hazard modeling method was applied for multivariate analysis of OS.

RESULTS

Patient's Characteristics

Fifty-seven patients were enrolled between March 2006 and January 2009. The median age of enrolled patients was 64 years (range 28 to 84 y) at diagnosis, and the male/female ratio was 1.6/1.0. The median ECOG PS was 1 (range, 0 to 2). Twenty-five patients (43.9%) were never smoker and 40 of 57 patients (70.2%) had histologic subtype of adenocarcinoma. Table 1 presented the baseline characteristics of all patients. Approximately half of patients (54.4%) had more than 2 metastatic lesions and major involved organs were bone and lung. Eighteen of 57 patients had only BSC without chemotherapy. Their median PS was ECOG 1 (range, 1 to 2). Therefore, we recommended these patients systemic chemotherapy to improve survival time and quality of life. However, they refused chemotherapy and wanted instead to receive only BSC. Platinum-based doublet chemotherapy, including paclitaxel, docetaxel, vinorelbine, and gemcitabine, was administered as first-line chemotherapy to 35 of 39 patients (90%) and 4 of 39 patients (10%) received EGFR-TKIs. During the follow-up, 22 patients received EGFR-TKIs as any line.

Sensitivity and Specificity of Detection for EGFR Mutation in Serum DNA

In pairs of tumor and serum samples from 57 patients, the concordance of EGFR mutation positive between tumor and serum samples was detected in 8 pairs. EGFR mutations were detected in the serum samples of 11 patients and in the tumor samples of 12 patients. We detected 11 EGFR exon 19 (7 patients) or 21 (4 patients) mutations in serum samples. In tumor samples, there were 12 EGFR exon 19 (8 patients) or 21 (4 patients) mutations. EGFR mutation status was consistent in

TABLE 3. Clinical Features and EGFR Mutations (N=57)

	Tumor Samples			Serum Samples		
	Positive (%)	Negative (%)	P	Positive (%)	Negative (%)	P
Age (y)						
Younger than or equal to 65 (N=31)	6 (19.4)	25 (80.6)	0.731	7 (22.6)	24 (77.4)	0.493
Older than 65 (N=26)	6 (23.0)	20 (77.0)		4 (15.4)	22 (84.6)	
Sex						
Male (N=35)	4 (11.4)	31 (88.6)	0.043	4 (11.4)	31 (88.6)	0.049
Female (N=22)	8 (36.4)	14 (63.6)		7 (31.8)	15 (68.2)	
Smoking status						
Current or ever (N=32)	3 (9.3)	29 (90.7)	0.014	3 (9.4)	29 (90.6)	0.044
Never (N=25)	9 (36.0)	16 (64.0)		8 (32.0)	17 (68.0)	
Histology						
Adenocarcinoma (N=40)	10 (25.0)	30 (75.0)	0.315	10 (25.0)	30 (75.0)	0.146
Squamous cell carcinoma (N=17)	2 (11.8)	15 (88.2)		1 (5.9)	16 (94.1)	
Disease status						
3B (N=7)	2 (28.6)	5 (71.4)	0.630	2 (28.6)	5 (71.4)	0.610
4 (N=50)	10 (25.0)	40 (75.0)		9 (18.0)	41 (82.0)	
No. of metastatic sites						
0-1 (N=26)	4 (15.4)	22 (84.6)	0.336	4 (15.4)	22 (84.6)	0.493
≤2 (N=31)	8 (25.8)	23 (74.2)		7 (22.6)	24 (77.4)	
Response to EGFR-TKIs (N=22)						
Partial Response	6 (27.4)	0 (0.0)	<0.001	4 (18.3)	2 (9.1)	0.009
Stable disease	1 (4.5)	12 (54.5)		1 (4.5)	12 (54.5)	
Progressive Disease		3 (13.6)		0	3 (13.6)	

EGFR-TKIs indicates epidermal growth factor receptor tyrosine kinase inhibitors.

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50 of the 57 pairs (87.7%). There was a high correlation between the mutations detected in tumor sample and the mutations detected in the matched serum sample ($P < 0.001$; correlation index 0.62) (Table 2).

Correlation Between EGFR Mutation Status and Patient Characteristics

In tumor samples, EGFR mutations were observed significantly more frequently in female patients ($P = 0.043$) and never smokers (0.014). These findings were consistent in serum samples (female, $P = 0.049$; and never smokers, $P = 0.044$). Other patients' characteristics did not have the significant association with EGFR mutation status in both tumor and serum samples. In analysis for patients treated with EGFR-TKIs as any line, response for EGFR-TKIs had the close correlation to EGFR mutations in both tumor ($P < 0.001$) and serum ($P = 0.009$) samples (Table 3).

Correlation Between EGFR Mutations Status and Survival

There was no significant difference for OS in 57 patients according to EGFR mutations status in serum samples ($P = 0.440$, Fig. 1A). This finding was identical on analysis for EGFR mutations status in tumor samples ($P = 0.532$, Fig. 1B). Eighteen of 57 patients received only supportive care without chemotherapy or EGFR-TKIs. In analysis for 39 patients with chemotherapy, there was still no significant difference for OS according to EGFR mutation status in both serum and tumor samples. An association between EGFR mutation status and OS was not observed in either serum or tumor samples based analyses ($P = 0.887$, Fig. 1C). In subgroup analysis for patients treated with EGFR-TKIs as any line (N = 22), there was no significant difference for OS according to EGFR mutations status in serum, tumor, and either serum or tumor samples based analyses (Fig. 2A, B, and C).

EGFR Mutations in Both Serum and Tumor Samples as a Prognostic Factor

Univariate analysis revealed that decreased OS was significantly associated with ECOG PS of 2 or more and no receiving of EGFR-TKIs. In multivariate analysis, ECOG PS of 2 or more (hazard ratio of 0.414; 95% confidence interval, 0.184%-0.932%, $P = 0.033$), and no receiving of EGFR-TKIs (hazard ratio of 0.369: 95% confidence interval, 0.193%-0.706%, $P = 0.003$) were also significantly associated with decreased OS. EGFR mutations in both serum and tumor samples did not have the impact as a prognostic marker for survival (Table 4).

DISCUSSION

EGFR mutations as predictive marker for EGFR-TKIs in patients with NSCLC have been used widely.[12] In addition, although there is controversy, EGFR mutations have been

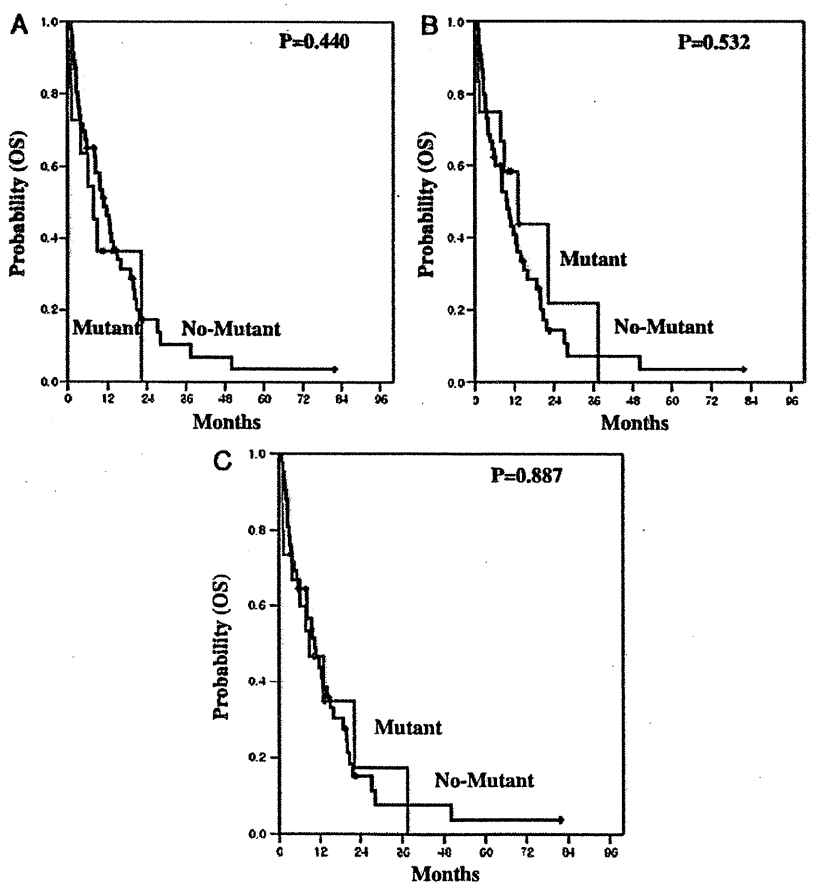

FIGURE 1. Kaplan-Meier probability of overall survival (OS) in all patients (N = 57). A, OS by epidermal growth factor receptor (EGFR) mutation status measured in serum DNA. B, OS by EGFR mutation status measured in tumor tissue. C, OS by EGFR mutation status measured in either tumor tissue or serum DNA.

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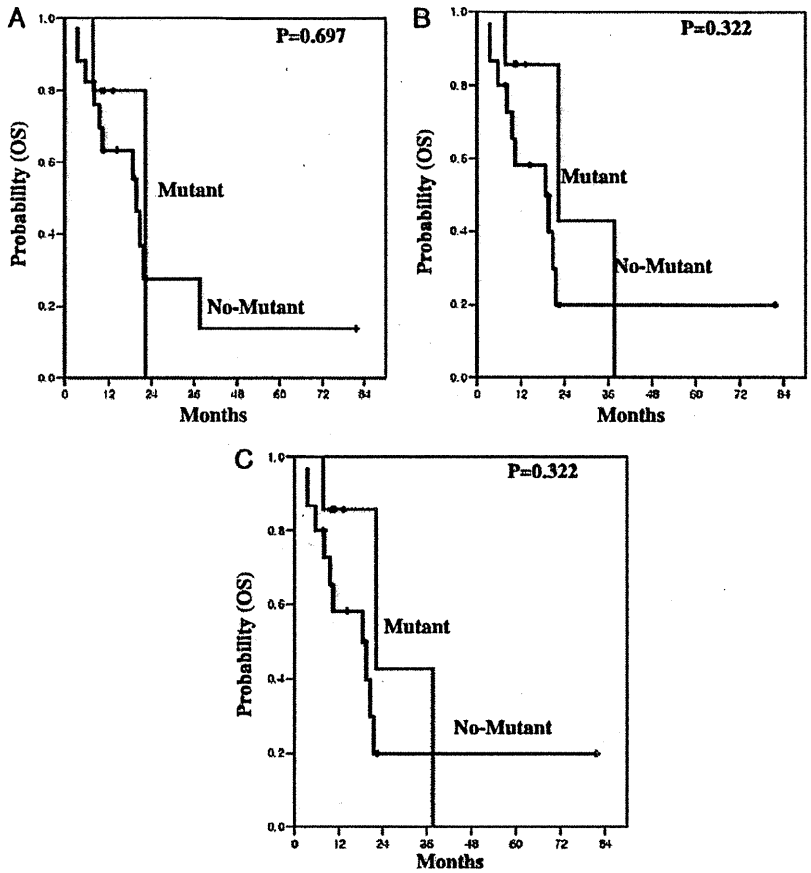

FIGURE 2. Kaplan-Meier probability of overall survival (OS) in patients receiving epidermal growth factor receptor (EGFR)-tyrosine kinase inhibitors (N = 22). A, OS by EGFR mutation status measured in serum DNA. B, OS by EGFR mutation status measured in tumor tissue. C, OS by EGFR mutation status measured in either tumor tissue or serum DNA.

considered as a good prognostic marker irrespective of use of EGFR-TKIs.[13,14] Thus, what we know the status of EGFR mutations is very important to identify patients who might derive more benefit from EGFR-TKIs and to make decision in clinical practice. Our results demonstrated that serum sample might be alternatively used in the difficult time of getting tumor tissue for analyzing the status of EGFR mutation status. There was a high correlation between the mutations detected in serum sample and the mutations detected in the matched tumor sample (correlation index 0.62; $P < 0.001$). EGFR mutation status was consistent in 50 of the 57 pairs (87.7%). Meanwhile, EGFR mutations were observed in either serum samples only or tumor samples only in 12.2% of our patients. This finding has also been observed in previous similar studies.[18,23] This inconsistency in mutation status is considered due to the heterogeneity of genetic abnormalities in tumors and limitation of detection method.

The response for EGFR-TKIs was significantly associated with EGFR mutation in both tumor samples and serum samples ($P < 0.05$). However, there was no significant differences in OS according to the status of EGFR mutations in both serum and tumor samples ($P > 0.05$). The role of EGFR mutations as a prognostic marker for survival in patients with NSCLC is still not proven. The Iressa NSCLC Trial Assessing Combination Treatment study did show a significant increased survival of EGFR mutation-positive patients treated with chemotherapy, irrespective of EGFR-TKI[13] treatment. These findings are also in agreement with the molecular analysis of a phase III trial of erlotinib (TRIBUTE) in which EGFR mutations seemed to be a positive prognostic indicator irrespective of EGFR-TKI treatment.[14] On the contrary, Kosaka et al[24,25] showed that EGFR mutation did not affect the prognosis for Asian patients with adenocarcinoma who were not treated with gefitinib and in primary resected NSCLC.[26] These discrepancies among studies including our study may be caused by inherent heterogeneity in NSCLC and the existence of confounding factors such as KRAS mutational status, and different clinical characteristics.

In this study, EGFR mutations were detected in the serum samples of 11 patients (19.3%) and in the tumor samples of 12 patients (21.0%). Response rate for EGFR-TKIs as any line was 27.3% (6 of 22 patients). These findings were consistent with previous reports. It has been reported that the pooled cumulative analysis of clinical data of EGFR-TKIs from Asian country consistently showed that EGFR-TKIs can achieve approximately 20% to 30% of response.[27] Recently, Ahn et al[28] reported that the incidence of EGFR mutations for all comers who were treated with erlotinib was higher (26.1%) in Korean patients when compared with western countries (< 10%) and Jang et al[29] showed that EGFR mutations were present in 24% of patients with adenocarcinoma of the lung. Generally, EFGR mutations are shown to be more prevalent in females, never smokers, patients of Asian ethnicity and those with histology of adenocarcinoma.[30] We also showed that the mutation frequencies were significantly higher in never

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TABLE 4. Prognostic Factors for OS in Multivariate Analysis (N = 57)

Variables	Overall Survival Mo (95% CI)	Univariate *P* value	Multivariate *P* value	HR (95% CI)
Sex				
Male	10.4 (6.93-13.88)	0.201		
Female	9.6 (3.44-15.82)			
Age (y)				
Younger than or equal to 65	12.4 (6.74-18.06)	0.094		
Older than 65	8.1 (3.71-12.49)			
Smoking history				
Current or ever	9.7 (3.34-16.06)	0.159		
Never	13.0 (5.82-20.18)			
ECOG PS				
0-1	11.7 (8.15-15.25)	0.010	0.033	0.414 (0.184-0.932)
≤2	2.1 (0.0-10.36)			
Histology				
Adenocarcinoma	9.6 (7.06-12.14)	0.620		
Squamous cell carcinoma	11.7 (1.08-22.32)			
Disease status				
3B	11.7 (4.51-18.89)	0.168		
4	9.7 (6.82-12.58)			
No. of metastatic sites				
0-1	10.4 (6.36-14.44)	0.698		
≤2	9.6 (4.13-15.07)			
Use of EGFR-TKIs during treatment				
Yes	20.1 (17.84-23.56)	<0.001	0.003	0.369 (0.193-0.706)
No	6.0 (1.06-10.94)			
Tumor-EGFR mutations				
Positive	13.0 (3.89-22.11)	0.553		
Negative	9.7 (6.92-12.48)			
Serum-EGFR mutation				
Positive	7.8 (2.41-13.19)	0.443		
Negative	10.8 (7.36-14.24)			

ECOG PS indicates Eastern Cooperative Oncology Group performance status; EGFR-TKIs, epidermal growth factor receptor tyrosine kinase inhibitors.

smokers and in female patients in both serum and tumor samples but not in histology of adenocarcinoma.

We used the PNA-LNA PCR clamp method as the detection test for EGFR mutations in serum sample. The usefulness of this method has been proven in previous studies.[19–22] With this method, EGFR mutations can be detected from small cytologic specimens such as those from bronchial washing, pleural effusions, sputum collection, which are frequently used for diagnosis of advanced NSCLC. Tanaka et al[20] reported that the sensitivity and the specificity of this test was 97% and 100%, respectively. Recently, Maemondo et al[22] used this method for detection of EGFR mutations in the phase III trial comparing gefitinib and standard carboplatin-paclitaxel chemotherapy for NSCLC with mutated EGFR. The results of the analyses could also be gained within several days, so the clinical decision is usually not delayed.

In our analysis, 2 patients with EGFR mutation-negative in serum samples showed objective response to EGFR-TKIs, whereas not in tumor samples. In addition, in the 'Table 2', 4 patients with EGFR mutation-negative in serum sample were revealed as EGFR mutation-positive from analysis using tumor samples. Thus, we need to develop a highly sensitive and precise methodology detecting the EGFR mutation in serum sample. Nevertheless, the inability to obtain primary tumor tissue, particularly through repeat biopsy, from patients with advanced stage lung cancer makes the use of serum as a surrogate sample for genetic analysis clinically important.

This study has some limitations such as small number of patients, heterogeneous patients' group, single institution study, and the use the different technique for both the serum and the tissue. We used PNA-LNA PCR clamp method for serum samples but genomic PCR/direct sequencing for tumor tissues. In other words, we used the different technique for analyzing EGFR mutation status in both the serum and the tissue. To verify the validity of the serum as a substitute for the tumor tissue, we should have used the same technique for both the serum and the tissue. The difference of technique might affect the result for the concordance of EGFR mutation in serum and tumor tissue. Nevertheless, our report suggested that serum sample might be alternatively used in the difficult time of getting tumor tissue for analyzing the status of EGFR mutation status in patients with advanced NSCLC. Future prospective studies with larger sample size and the same technique are needed to reaffirm the role of serum sample as a surrogate tissue for EGFR mutation analysis in NSCLC.

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Phase II Study of Gefitinib Readministration in Patients with Advanced Non-Small Cell Lung Cancer and Previous Response to Gefitinib

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Key Words
Epidermal growth factor receptor · Epidermal growth factor receptor-tyrosine kinase inhibitor · Gefitinib · Non-small cell lung cancer · Chemotherapy

Abstract
Objective: Salvage treatment for acquired resistance to gefitinib has yet to be developed. We conducted the first prospective phase II study of gefitinib readministration in previous gefitinib responders. ***Methods:*** Gefitinib (250 mg/day) was readministered to patients with advanced/metastatic non-small cell lung cancer who had achieved objective response to initial gefitinib and subsequently received cytotoxic chemotherapy after disease progression with initial gefitinib. The primary endpoint was the objective response rate with gefitinib readministration. Secondary endpoints were disease control rate, progression-free survival (PFS), overall survival (OS), quality of life, and toxicity. Changes in lung cancer-related symptoms were evaluated using the seven-item lung cancer subscale of the questionnaire. ***Results:*** Sixteen patients were enrolled between February 2005 and January 2008. Most had received ≥3 regimens of chemotherapy. Response and disease-control rates for all patients were 0 and 44%. Median PFS and OS were 2.5 and 14.7 months, respectively. Four of 7 patients with stable disease experienced a long duration (≥6 months) of disease control without severe toxicity. Symptom improvement was observed in 2 of 12 patients (17%) for whom quality of life was evaluable. ***Conclusion:*** Gefitinib represents a useful therapeutic option for selected previous gefitinib responders.
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Introduction

Gefitinib is the first commercially available epidermal growth factor receptor (EGFR)-tyrosine kinase inhibitor (TKI) and is widely used for the treatment of advanced or recurrent non-small cell lung cancer (NSCLC). The Iressa Pan-Asia Study (IPASS) demonstrated superior

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progression-free survival (PFS) in the gefitinib arm than in the carboplatin and paclitaxel arm for chemotherapy-naive patients with never-smoker or light-smoking status [1]. For EGFR mutation-positive patients, gefitinib monotherapy can produce superior PFS than carboplatin and paclitaxel or cisplatin and docetaxel combinations in the first-line setting [2, 3]. As a second-line therapy, gefitinib showed significantly better overall survival (OS) than placebo for never-smokers and patients of Asian origin in the Iressa Survival Evaluation in Lung Cancer (ISEL) trial and noninferiority of OS compared to docetaxel in the Iressa NSCLC Trial Evaluating Response and Survival versus Taxotere (INTEREST) study [4, 5].

Despite the initial efficacy of gefitinib monotherapy, acquired resistance appears almost inevitable and median PFS does not exceed 12 months [6]. Approximately 60–70% of cases with acquired resistance to EGFR-TKI can be explained by the secondary resistance T790M mutation [7, 8], acquired amplification of the MET oncogene [9, 10], or a small number of other secondary mutations, such as L858R-D761Y [11], L858R-L747S [12] and L858R-T854A [13]. Details of resistance have yet to be completely clarified, but establishment of salvage treatment is an urgent issue.

Several case reports have described successful readministration of gefitinib to NSCLC patients who achieved objective response with the initial administration of gefitinib before eventual progression [14, 15]. The present study represents the first prospective phase II study to evaluate gefitinib readministration as a therapeutic option for heavily pretreated patients with NSCLC who responded to initial gefitinib treatment and received subsequent cytotoxic chemotherapy.

Patients and Methods

Patient Eligibility

Subjects were patients with recurrent or metastatic NSCLC with documented progressive disease (PD) according to Response Evaluation Criteria in Solid Tumors (RECIST) [16] after achieving objective response with initial gefitinib and then receiving at least one subsequent cytotoxic chemotherapy regimen. Other eligibility criteria included an Eastern Cooperative Oncology Group performance status (PS) of 0–2, at least one unidimensionally measurable lesion, and adequate organ functions. Patients were excluded if they displayed unresolved chronic toxicity of prior therapy, other active malignancies, uncontrolled brain metastasis, or severe comorbidities. The institutional review board at each participating hospital approved all study protocols and the genetic analysis of tumors, and written informed consent was obtained from all patients prior to enrolment.

Treatment Plan

Patients received gefitinib at 250 mg/day. In the event of unacceptable toxicity defined as grade 3 or more, gefitinib was stopped until the toxicity resolved and improved to below grade 3 within 2 weeks. No dose reduction was permitted. Treatment was continued until disease progression, intolerable toxicity, or withdrawal of consent.

Evaluation of Response and Adverse Events

Evaluations of treatment response by computed tomography were repeated every 4 weeks according to RECIST. The minimum interval to qualify for stable disease (SD) was defined as 8 weeks. Responses were evaluated by the physician in charge and confirmed by extramural review. In addition, changes in lung cancer-related symptoms were evaluated using the seven-item lung cancer subscale (LCS) of the questionnaire [17]. The LCS is an independently validated tool that measures disease-related symptoms of lung cancer on a scale of 0 (most symptomatic) to 28 (asymptomatic). A change of ≥ 2 points in LCS score reportedly reflects a minimally important difference associated with PS, weight loss, objective tumor response, and time to progression [17]. Toxicity was graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events v3.0 (CTCAE v3.0).

Statistical Analysis

Objective response rate (RR) with gefitinib readministration was taken as the primary endpoint. Secondary endpoints were disease control rate (DCR), PFS, OS, symptom improvement rate, time to symptom improvement, and toxicity. DCR was defined as the sum of the RR plus the rate of SD. Simon's two-stage minimax design was used to determine the sample size and decision criteria for this phase II study. With a target activity level of 25% (P1) and the minimum RR of interest set at 5% (P0), 14 evaluable patients were needed to accept the hypothesis and a 5% significance level to reject the hypothesis with 80% power. Assuming an inevaluability rate of $\leq 15\%$, we projected an accrual of 16 patients. All patients who were enrolled and treated with gefitinib were included in both efficacy and toxicity analyses. OS was defined as the interval between enrolment in this study and death from any cause. PFS was defined as the interval between enrolment in this study and the date of documented PD or death from any cause. If a patient was lost to follow-up, that patient was censored at the last date of contact. Median OS and PFS were estimated using the Kaplan-Meier analysis. Factors potentially associated with long SD were assessed as follows. Categorical variables were compared using Fisher's exact test or the χ^2 test, while continuous variables were assessed using the Mann-Whitney nonparametric test. Relevant parameters for influence on long SD were studied by univariate analysis using the log-rank test. Differences were considered to be significant at the level of $p < 0.05$. Statistical analysis was performed using JMP 8 software (SAS Institute, Cary, N.C., USA).

Results

Patient Characteristics

Between February 2005 and January 2008, a total of 16 patients were enrolled in this study. Patient characteristics are described in table 1. The major tumor histological

Table 1. Patient characteristics (n = 16)

Characteristics	n (%)
Age, years	
Median	66.5
Range	53–79
Sex	
Male	3 (19)
Female	13 (81)
ECOG PS	
0	5 (31)
1	9 (56)
2	2 (13)
Histology	
Adenocarcinoma	14 (88)
Squamous cell carcinoma	1 (6)
Large-cell carcinoma	1 (6)
Smoking history	
Current or ex-smoker	5 (31)
Never-smoker	11 (69)
Stage	
IIIB	1 (6)
IV	10 (63)
Recurrence	5 (31)
EGFR mutation	
Positive	3 (19)
Negative	3 (19)
Unknown	10 (63)

ECOG = Eastern Cooperative Oncology Group.

Table 2. Summary of prior therapy for NSCLC (n = 16)

Characteristics	n (%)
Number of prior chemotherapy regimens	
2	2 (13)
3	9 (56)
4	2 (13)
5	2 (13)
6	1 (6)
Best response to prior cytotoxic chemotherapy	
Partial response	6 (38)
SD	7 (44)
PD	3 (19)
Time from first-line treatment to readministration of gefitinib	
≤12 months	2 (13)
12–24 months	4 (26)
≥12 months	10 (63)
Period of initial gefitinib administration	
≤6 months	1 (6)
6–12 months	7 (44)
≥12 months	8 (50)
Time from last day of initial gefitinib administration to first day of gefitinib readministration	
≤6 months	8 (50)
6–12 months	6 (38)
≥12 months	2 (13)

type was adenocarcinoma in 14 patients (88%). Eleven patients (69%) were never-smokers. Three patients showed EGFR gene mutations (2 patients with exon 19 deletions; 1 patient with L861Q in exon 21), 3 had the wild-type gene, and the status of the remaining 10 patients was unknown. All mutational analyses were performed using biopsy specimens obtained before initial gefitinib treatment.

All patients had received various therapies before study entry (table 2). Fourteen patients received gefitinib readministration as a fourth-line or later therapy.

Tumor Response and Survival

Responses were evaluable for 15 of the 16 enrolled patients. No patients achieved an objective response, with an overall RR of 0% [95% confidence interval (CI), 0–21%], while 7 patients (44%) showed SD and 8 patients (50%) had PD as the best response. DCR was 44% (95% CI, 20–70%). One patient experienced a transient reduction in diameter of the primary lesion. However, due to regrowth of other metastasis, the best response of this patient was SD (fig. 1). By the time of analysis, all patients had experienced disease progression and 14 patients had died. With a median follow-up of 14.7 months, median PFS and OS were 2.5 months (95% CI, 1.6–3.2 months) and 14.7 months (95% CI, 11.1–15.5 months), respectively (fig. 2). Four of 7 patients with SD experienced a long duration (≥6 months) of disease control. When we compared baseline characteristics between patients with and without long SD (≥6 months), no significant differences were observed in age, sex, PS, histology, smoking history, number of previous treatment regimens, duration of initial gefitinib treatment, or interval between initial and rechallenge of gefitinib (table 3). One of the 3 patients with EGFR gene mutations (L861Q) had SD with 6.7 months of PFS, while the other 2 patients had PD as the best response.

Toxicity Profile

Toxicity was evaluated in all eligible patients. The most common adverse event was fatigue in 13 patients (81%), including 2 patients with grade 3. One patient experienced grade 4 central nervous system cerebrovascu-

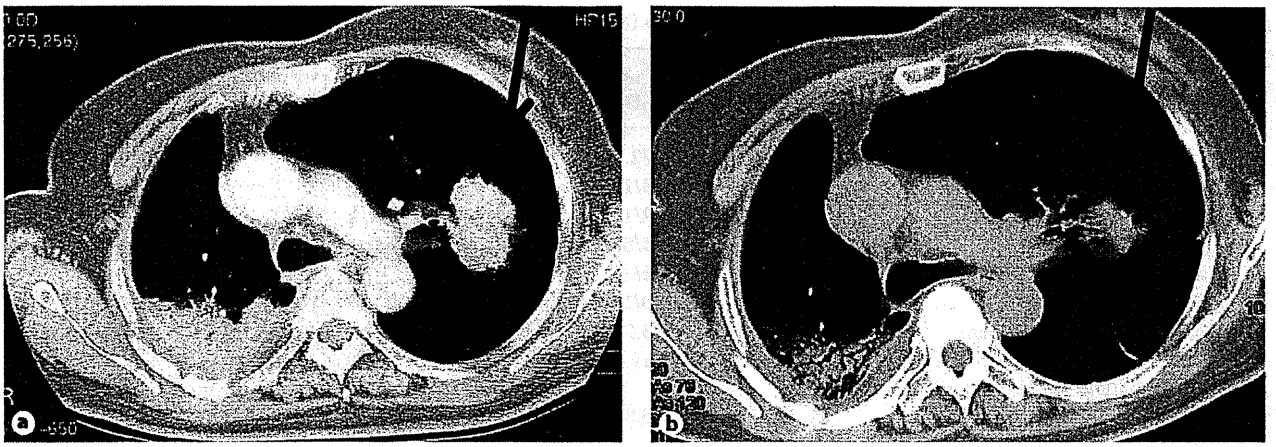

Fig. 1. Primary lesion in patient 5 (arrow) before (**a**) and 45 days after (**b**) gefitinib readministration.

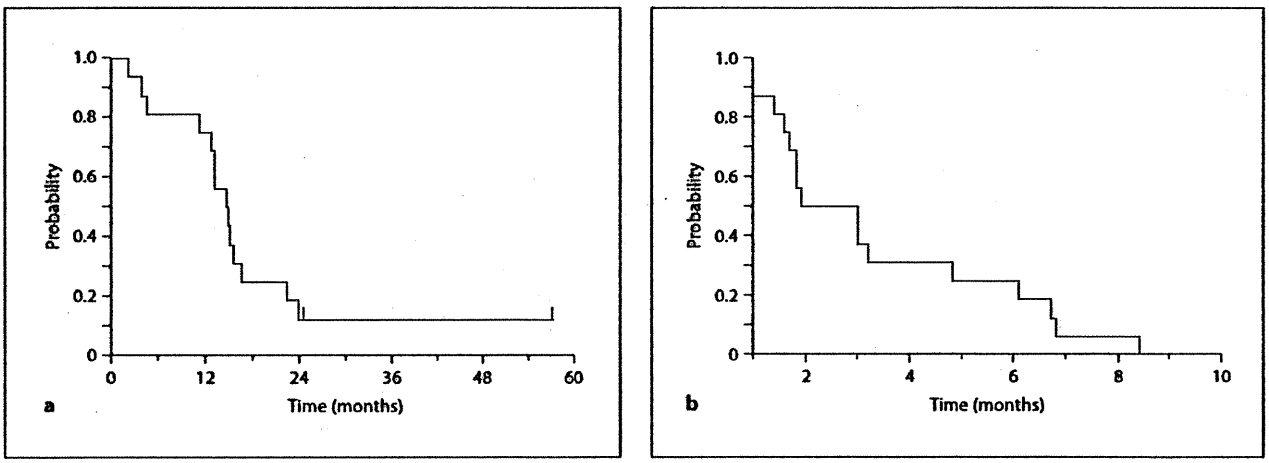

Fig. 2. OS (**a**) and PFS (**b**) for all eligible patients (n = 16) calculated according to the Kaplan-Meier method. Median survival time was 14.7 months (95% CI, 11.1–15.5 months) and median PFS was 2.5 months (95% CI, 1.6–3.2 months).

lar ischemia and terminated gefitinib treatment on day 47. Overall, toxicity appeared to be similar to the previously published trials of gefitinib monotherapy.

Symptom Improvement

LCS was evaluated in 12 of the 16 enrolled patients and compliance rate (ratio of the number of assessable weekly forms to the number of forms expected) was 70%. Median baseline LCS was 22 (range 12–28). Symptom improvement was observed in 2 of 12 patients, providing a symptom improvement rate of 16.7% (95% CI, 2.1–48.4%). Time to symptom improvement in these 2 patients was 1 and 4 weeks [17].

Discussion

To the best of our knowledge, this represents the first prospective phase II study to assess whether gefitinib readministration confers any clinical benefit in patients

Table 3. Comparison between patients with or without long duration (≥6 months) of SD

Characteristics/groups	Patients with long SD (n = 4)	Patients without long SD (n = 12)	p
Mean age ± SD, years	72.5 ± 3.9	64.5 ± 2.3	0.10
Sex (male/female)	3/1	10/2	1.00
ECOG PS (0/1/2)	2/1/1	3/8/1	0.33
Histology (Ad/Sq/La)	4/0/0	10/1/1	0.68
Smoking history (ever/never)	2/2	3/9	0.55
Stage (IIIB/IV/Rec)	0/1/3	1/9/2	0.09
Mean number of previous regimens	3.5	3.4	0.90
Median duration of initial gefitinib treatment, months	19.4	10.6	0.59
Median interval between initial and rechallenge gefitinib administrations, months	8.8	5.5	0.10
MST of gefitinib rechallenge, months	NR	12.8	0.03

Ad = Adenocarcinoma; Sq = squamous cell carcinoma; La = large-cell carcinoma; Rec = recurrence; MST = median survival time; NR = not reached.

with advanced NSCLC who have previously achieved objective response with the initial administration of gefitinib. No patients exhibited objective response, the primary endpoint of this study, suggesting that gefitinib readministration has little effect with respect to tumor shrinkage. However, the fact that 4 patients achieved a long duration (≥6 months) of disease control without severe toxicity is noteworthy.

Several retrospective studies have described the clinical activity of one EGFR-TKI treatment after the failure of another [18–24] or readministration of the same drug [14, 15, 25]. Most such reports have noted favorable results, although Viswanathan et al. [19] and Costa et al. [24] reported no or only a limited response to erlotinib after progression on gefitinib. Two prospective studies by Cho et al. [26] and Lee et al. [27] have shown results similar to our own, namely that RR/DCR were 9.5%/28.6% and 4.3%/8.7% each. In another prospective study, Riely et al. [28] also reported that in patients who develop acquired resistance, stopping gefitinib or erlotinib results in symptomatic progression, worsening of results on FDG-PET, and increased tumor size, while restarting EGFR-TKI results in a median 1% decrease in tumor diameter, 4% decrease in FDG-PET uptake and improvement of symptoms. These results imply that some patients with clinically acquired resistance to EGFR-TKI possess some tumor cells that remain sensitive to EGFR blockade and may benefit from readministration of EGFR-TKI.

Identifying the predictive factors to distinguish those who might benefit from gefitinib readministration is also an important issue. Tomizawa et al. [25] mentioned the importance of the 'EGFR-TKI-free interval'. This retrospective study of gefitinib readministration demonstrated a favorable result, with RR 25% and DCR 65%, accompanying a sufficient EGFR-TKI-free interval (median 217 days) with 1–3 regimens of cytotoxic chemotherapy in all patients [25]. Conversely, Costa et al. [24] reported that erlotinib was ineffective (RR 6%; DCR 22%) in 18 patients with resistance to gefitinib without any interval after resistance to gefitinib. In the present study, due to the lack of a control group (i.e. cohort of patients who did not have any gefitinib readministration), we could only examine the prognostic factors for patients retreated with gefitinib. No significant differences were seen regarding baseline characteristics (including EGFR-TKI-free interval) between patients with long SD (n = 4) and without long SD (n = 12). This may, in part, be attributed to the small sample size.

Some authors have explained the usefulness of EGFR-TKI readministration with the hypothesis that cytotoxic chemotherapy administered after the initial EGFR-TKI might modify the proportion of sensitive or resistant cells or produce some genetic changes in the tumor [14, 15, 25]. We could not perform comparative molecular analysis of tissue specimens between before initial administration and readministration of gefitinib. Further investigations are required regarding this issue.

In conclusion, gefitinib readministration seems to represent a potential therapeutic option for some selected NSCLC patients who respond to the initial gefitinib therapy. New approaches for identifying molecular markers are important to overcome the resistance to EGFR-TKIs seen with progression after initial response.

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Disclosure Statement

The authors have no conflicts of interest to declare.

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CANCER

Optimization of Dosing for EGFR-Mutant Non–Small Cell Lung Cancer with Evolutionary Cancer Modeling

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Non–small cell lung cancers (NSCLCs) that harbor mutations within the epidermal growth factor receptor (*EGFR*) gene are sensitive to the tyrosine kinase inhibitors (TKIs) gefitinib and erlotinib. Unfortunately, all patients treated with these drugs will acquire resistance, most commonly as a result of a secondary mutation within *EGFR* (T790M). Because both drugs were developed to target wild-type EGFR, we hypothesized that current dosing schedules were not optimized for mutant EGFR or to prevent resistance. To investigate this further, we developed isogenic TKI-sensitive and TKI-resistant pairs of cell lines that mimic the behavior of human tumors. We determined that the drug-sensitive and drug-resistant *EGFR*-mutant cells exhibited differential growth kinetics, with the drug-resistant cells showing slower growth. We incorporated these data into evolutionary mathematical cancer models with constraints derived from clinical data sets. This modeling predicted alternative therapeutic strategies that could prolong the clinical benefit of TKIs against EGFR-mutant NSCLCs by delaying the development of resistance.

INTRODUCTION

Gefitinib (Iressa) and erlotinib (Tarceva) are first-generation epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKIs) that were designed as adenosine triphosphate (ATP) mimetics to block wild-type receptor activity. While being developed, these drugs were serendipitously found to be most clinically effective against those non–small cell lung cancers (NSCLCs) that harbor mutations in exons encoding the kinase domain of EGFR (*1*–*3*). Common alterations include small in-frame deletions in exon 19 (19 dels) and a point mutation within exon 21 (L858R), both of which lead to sustained activity of the kinase (*4*–*6*). More than 70% of patients with *EGFR*-mutant tumors treated prospectively with either TKI show tumor volume decreases, with an overall median survival of ~30 months (*7*–*9*).

Unfortunately, lung tumors in all patients eventually develop acquired resistance (*7*, *10*). The most common mechanism of resistance is a second site mutation within exon 20 of *EGFR* (T790M), observed in ~50% of cases (*11*, *12*). This change leads to altered binding of the drug within the ATP pocket (*13*).

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Currently, targeted therapeutic options for T790M-harboring NSCLCs are limited. Second-generation EGFR TKIs [for example, HKI-272 (neratinib) and BIBW-2992 (afatinib)] are more potent than gefitinib and erlotinib against EGFR T790M (*14*, *15*). However, because they inhibit drug-sensitive mutants at lower doses than they inhibit the T790M mutant, they still select for T790M-harboring clones in models of acquired resistance in vitro (*14*). Their antitumor activity in patients with acquired resistance to gefitinib and erlotinib has been disappointing (*16*, *17*).

We hypothesized that, because clinically available EGFR TKIs were developed against wild-type EGFR, current empiric dosing regimens were not optimally designed to inhibit the *EGFR* mutants in NSCLC nor to minimize the development of drug resistance. Here, we have identified differences in the growth kinetics of TKI-sensitive and TKI-resistant (T790M-containing) isogenic NSCLC cells. We incorporated these findings, along with patient data, into evolutionary cancer models (*18*) to generate mathematical models predictive of tumor behavior. This approach identified several strategies to improve the treatment of EGFR-mutant NSCLC before and after the emergence of T790M-mediated acquired resistance.

RESULTS

Derivation of *EGFR*-mutant TKI-resistant lung adenocarcinoma cells

To determine the physical characteristics of TKI-sensitive and TKI-resistant cells, we derived in vitro cellular models of T790M-mediated resistance using EGFR-mutant TKI-sensitive PC-9 cells (19 del), well-established TKI dose-escalation protocols (*14*, *19*, *20*), and either a reversible quinazoline (erlotinib) or an irreversible quinazoline (BIBW-2992) that binds covalently to C797 in EGFR. After 120 days in culture, PC-9 cells resistant to erlotinib and BIBW-2992 emerged that grew in drug concentrations ~100-fold (5 μM) and ~1000-fold (500 nM) the initial IC_{50} (median inhibitory concentration), respectively, of the

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