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Mutational status of *EGFR* and *KIT* in thymoma and thymic carcinoma

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Summary This study was conducted to evaluate the prevalence of *EGFR* and *KIT* mutations in thymomas and thymic carcinomas as a means of exploring the potential for molecularly targeted therapy with tyrosine kinase inhibitors. Genomic DNA was isolated from 41 paraffin-embedded tumor samples obtained from 24 thymomas and 17 thymic carcinomas. *EGFR* exons 18, 19, and 21, and *KIT* exons 9, 11, 13, and 17, were analyzed for mutations by PCR and direct sequencing. Protein expression of EGFR and KIT was evaluated immunohistochemically. *EGFR* mutations were detected in 2 of 20 thymomas, but not in any of the thymic carcinomas. All of the *EGFR* mutations detected were missense mutations (L858R and G863D) in exon 21. EGFR protein was expressed in 71% of the thymomas and 53% of the thymic carcinomas. The mutational analysis of *KIT* revealed only a missense mutation (L576P) in exon 11 of one thymic carcinoma. KIT protein was expressed in 88% of the thymic carcinomas and 0% of the thymomas. The results of this study indicate that *EGFR* and *KIT* mutations in thymomas and thymic carcinomas are rare, but that many of the tumors express EGFR or KIT protein.

1. Introduction

Thymic epithelial tumors are uncommon neoplasms and there are two major histological types: thymoma and thymic carcinoma [1]. Surgical resection is the preferred treatment option for all subtypes of thymoma and thymic carcinoma. However, thymic carcinomas and some thymomas tend to behave in a malignant manner clinically, and in many cases dissemination or distant metastasis has already occurred at presentation. Patients with metastatic or unresectable tumors are candidates for systemic chemotherapy, but no standard chemotherapy has been established because of the rarity of both tumors [2–5], and alternative therapeutic molecular targets are needed.

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Receptor tyrosine kinases such as epidermal growth factor receptor (EGFR) and KIT, contribute to a number of processes related to the survival and growth activity of many solid tumors, making them promising targets for cancer therapy [6–8]. Recent studies have shown that the presence of kinase domain mutations in the EGFR gene in non-small cell lung cancer (NSCLC) tissue predicts a significant clinical response to small-molecule tyrosine kinase inhibitors (TKIs) of EGFR, such as gefitinib and erlotinib [9], and it is widely known that there is an association between exon 11 mutations of the KIT gene in gastrointestinal stromal tumors (GISTs) and greater responsiveness to imatinib as a small-molecule TKI of KIT [10].

Several immunohistochemical studies have shown overexpression of EGFR protein in both thymoma and thymic carcinoma [11,12], and in thymic carcinoma, immunohistochemical studies have shown a high frequency of KIT overexpression but that thymomas express hardly any KIT [13,14]. Two interesting cases have recently been reported. One was a case of thymic carcinoma with an activating *KIT* mutation that responded to imatinib, reported by Strobel et al. [15], and the other was a case of thymic carcinoma with *EGFR* mutations that was responsive to gefitinib, reported by Yamaguchi et al. [16]. However, because of the rarity of these tumors, information on the mutational status of *EGFR* and *KIT* in thymomas and thymic carcinomas has been limited to only a few reports, and the prevalence of *EGFR* and *KIT* mutations remains unknown.

In this study, we investigated the status of *EGFR* and *KIT* mutations in thymoma and thymic carcinoma patients to explore the potential for molecularly targeted therapy with TKIs. We also investigated the relation between protein expression assessed by immunohistochemistry and the mutational status of *EGFR* and *KIT*.

2. Patients and methods

2.1. Patients

The tumor samples used in this study were obtained from paraffin-embedded surgical specimens from 41 cases of thymoma or thymic carcinoma treated surgically at the National Cancer Center Hospital East between 1993 and 2005. All samples were reviewed to confirm the diagnosis of thymoma or thymic carcinoma. The clinical data of all patients was collected from their medical records. This study was approved by the Institutional Review Board of our institution.

The characteristics of all of the patients are listed in Table 1. Patient age ranged from 21 to 77 years, and their median age was 61 years. The specimens used were from 24 thymomas and 17 thymic carcinomas. According to the World Health Organization (WHO) classification of thymic epithelial tumors, the histological subtype of the thymomas was type A in 7 cases, type AB in 7 cases, type B1 in 6 cases, and type B2 in 4 cases. The histological subtype of the thymic carcinomas was squamous cell carcinoma in 14 cases, and adenocarcinoma, adenosquamous carcinoma, and nonspecified in 1 case each. According to the system described by Masaoka et al. [17], the clinical stage was stage I in 15 patients, stage II in 8 patients, stage III in 9 patients, stage IVa in 1 patient, and stage IVb in 8 patients. All patients had undergone total resection ($n = 36$) or partial resection ($n = 5$) after obtaining their informed consent in accordance with institutional guidelines.

Table 1 Patient characteristics

	Patients ($n = 41$)
Age, years	
Median	61
Range	21–77
Gender	
Female	20
Male	21
Histology	
Thymoma	24
Thymic carcinoma	17
Stage	
I	15
II	8
III	9
IVa	1
IVb	8
Surgical procedure	
Total resection	36
Partial resection	5
Smoking history	
Never	19
Former	11
Current	11

2.2. Mutational analysis of *EGFR* and *KIT*

Tumor genomic DNA was isolated from paraffin-embedded samples of a total of 41 tumors, 24 thymomas and 17 thymic carcinomas. To ensure that tumor-cell-rich areas of tissues were isolated, hematoxylin and eosin stained slides were prepared from each selected paraffin-embedded block. Polymerase chain reaction (PCR) was performed to amplify exons 18, 19, and 21 of *EGFR* and exons 9, 11, 13, and 17 of *KIT* by using previously described primers [9,18], and the PCR products were directly sequenced with an ABI 3100 DNA Sequencer (Applied Biosystems, Foster City, CA, USA). All sequencing reactions were performed in both forward and reverse directions. A series of mutational analyses was performed at Mitsubishi Chemical Safety Institute Ltd.

2.3. Immunohistochemistry

Protein expression of EGFR and KIT was evaluated immunohistochemically in representative paraffin-embedded sections. EGFR staining was performed by using the DAKO (Carpinteria, CA, USA) pharmDX kit for EGFR according to the manufacturer's instructions, and immunostaining for KIT was performed by using a polyclonal rabbit antibody (A 4502; Dako, Glostrup, Denmark) according to the manufacturer's instructions. Staining of both markers was considered posi-

tive if more than 50% of the tumor cells stained. All slides were examined and scored independently by two observers (G.I. and K.Y.).

2.4. Statistical analysis

The variables measured in the study were tested for associations by Fisher's exact test. P values <0.05 were considered statistically significant.

3. Results

3.1. EGFR analysis of thymomas and thymic carcinomas

Sequencing of the *EGFR* tyrosine kinase domain encoded by exons 18, 19, and 21 was successful in 29 of the 41 tumors (Table 2). *EGFR* mutations were detected in 2 of the 20 thymomas, but direct sequencing showed no evidence of mutations in any of the 9 thymic carcinomas. All of the *EGFR* mutations detected were missense mutations in exon 21 (L858R or G863D), and no mutations were detected in exons 18 and 19. Examination of 21 thymomas and 17 thymic carcinomas for EGFR protein expression by immunohistochemistry revealed EGFR expression in 15 (71%) of the 21 thymomas and 9 (53%) of the 17 thymic carcinomas. The difference in EGFR expression between the thymomas and thymic carcinomas was not significant ($P=0.31$).

3.2. KIT analysis of thymomas and thymic carcinomas

It was possible to analyze the *KIT* mutation status of 22 thymomas and 11 thymic carcinomas by direct sequencing (Table 3). A missense mutation in exon 11 (L576P) was found in only one thymic carcinoma, and direct sequencing of *KIT* exons 9, 13, and 17 revealed no mutations in any of the tumors analyzed. Immunohistochemistry showed KIT protein expression in 15 (88%) of the 17 thymic carcinomas, but no KIT expression in any of the 24 thymomas ($P<0.0001$).

Table 4 summarizes the data of all patients whose tumors were positive for *EGFR* or *KIT* mutations. Exon 21 mutations in the *EGFR* gene were found in two thymomas (Fig. 1A and B), and an exon 11 mutation was identified in the *KIT* gene of 1 thymic carcinoma (Fig. 1C). Because these mutations were not detected in the normal lung tissues from the same patients, they were considered to be somatic mutations. Both patients whose tumors were positive for *EGFR* mutation were never smokers. All three patients had undergone surgical resection, and they are currently alive and relapse-free.

Table 2 EGFR status of thymomas and thymic carcinomas

EGFR mutation	Thymoma ($n=20$)	Thymic carcinoma ($n=9$)	
Exon 18	0	0	
Exon 19	0	0	
Exon 21	2	0	
No mutation	18	9	
EGFR expression	Thymoma ($n=21$)	Thymic carcinoma ($n=17$)	P
Positive	15 (71%)	9 (53%)	0.31

Table 3 KIT status of thymomas and thymic carcinomas

KIT mutation	Thymoma ($n=22$)	Thymic carcinoma ($n=11$)	
Exon 9	0	0	
Exon 11	0	1	
Exon 13	0	0	
Exon 17	0	0	
No mutation	22	10	
KIT expression	Thymoma ($n=24$)	Thymic carcinoma ($n=17$)	P
Positive	0 (0%)	15 (88%)	<0.0001

4. Discussion

In this study, *EGFR* mutations were observed in the DNA sequences of 2 thymomas of 29 tumors analyzed, and analysis of the *KIT* mutation status of 22 thymomas and 11 thymic carcinomas by direct sequencing revealed a missense mutation in exon 11 in only 1 thymic carcinoma. By contrast, 71% of the thymomas and 53% of the thymic carcinomas expressed EGFR protein, and overexpression of KIT was observed in 88% of the thymic carcinomas and 0% of the thymomas. The results show that the EGFR and KIT protein expression in the thymomas and thymic carcinomas was not associated with *EGFR* or *KIT* mutations.

A review of the medical literature retrieved reports of two studies that investigated *EGFR* mutations in thymomas or thymic carcinomas [19,20] and of one study that tested thymic carcinomas for *KIT* mutations [13]. Suzuki et al. reported that direct sequencing did not reveal any *EGFR* missense mutations in a total of 38 thymoma samples obtained from Japanese patients [19]. Meister et al. reported detecting no mutations in the tyrosine kinase domain of *EGFR* in 20 DNA samples from 17 thymomas and 3 thymic carcinomas analyzed by direct sequencing [20]. Pan et al. performed a mutation analysis of *KIT* by direct DNA sequencing in 21 thymic carcinomas, but found none [13]. To date, *EGFR* mutations (double missense mutations: G719A in exon 18 and L858R in exon 21) have been reported in one case of thymic carcinoma [16], and a *KIT* mutation (V560del in exon 11) in one case of thymic carcinoma [15]. The results of our study and review of the literature suggest that *EGFR* or *KIT* mutations are rare in thymomas and thymic carcinomas but that expression of EGFR and KIT is frequently present. Mutations that activate receptor tyrosine kinases contribute to the development of human carcinomas, and the activation of a mutation in the *KIT* gene is thought to be the most important factor in the pathogenesis of GISTs [7,8]. However, we speculate that *EGFR* or *KIT* mutations may not be implicated in the carcinogenesis of thymomas and thymic

Table 4 Summary of thymoma and thymic carcinoma patients with *EGFR* or *KIT* mutations in their tumors

Clinical characteristics						Mutation				IHC
No.	Age/sex	Smoking status	Masaoka stage	Histology	Clinical outcome	Gene	Exon	Nucleotide change	Amino acid change	
1	65/F	Never	II	Thymoma (type A)	3 years of disease-free survival after complete resection	*EGFR*	21	2573T > G	L858R	EGFR (+)
2	69/F	Never	III	Thymoma (type B1)	5 years of disease-free survival after complete resection	*EGFR*	21	2588G > A	G863D	EGFR (−)
3	59/M	Former (20 pack-years)	I	Thymic carcinoma (Sq)	6 years of disease-free survival after complete resection	*KIT*	11	1748T > C	L576P	KIT (+)

Abbreviations: Sq, squamous cell carcinoma; IHC, immunohistochemistry.

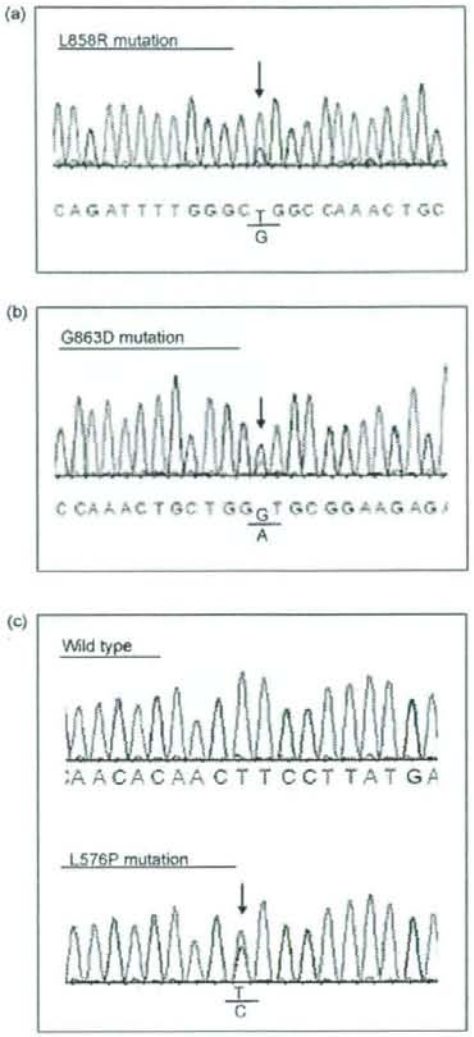

Fig. 1 Electropherograms of the products of direct sequencing of *EGFR* and *KIT*. (a and b) Two thymomas contained a single missense point mutation in exon 21 of *EGFR*. (c) One thymic carcinoma contained a single missense point mutation in exon 11 of *KIT*.

carcinomas because of the low frequency of *EGFR* or *KIT* mutations in these tumors.

Remarkably, the *EGFR* mutations (L858R and G863D, respectively, in exon 21) observed in the 2 thymomas in our study were similar to the active mutations in NSCLC that have been reported to be predictors of a therapeutic response to EGFR-TKI by NSCLCs [9,21]. Moreover, the *KIT* mutation (L576P in exon 11) identified in the 1 thymic carcinoma in our study had previously been described as one of the mutations that predicts a clinical response of GISTs to

imatinib [22]. We therefore speculate that patients whose thymoma or thymic carcinoma harbors *EGFR* or *KIT* mutations may profit from molecularly targeted therapy with a TKI of EGFR or KIT.

In conclusion, our findings indicate that somatic mutations of *EGFR* or *KIT* of the thymomas and thymic carcinomas are presented in a small number of patients. Further investigation is warranted to determine the susceptibility of such tumors to TKI therapy.

Conflict of interest

None declared.

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Efficacy and Safety of Erlotinib Monotherapy for Japanese Patients with Advanced Non-small Cell Lung Cancer

A Phase II Study

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Introduction: The aim of this study was to evaluate the efficacy and safety of Erlotinib in Japanese patients with previously treated non-small cell lung cancer (NSCLC). Available tumor biopsy samples were analyzed to examine relationships between biomarkers and clinical outcome.

Methods: This open-label phase II trial enrolled stage III/IV NSCLC patients who had progressive disease after at least one prior platinum-based chemotherapy regimen. Erlotinib was administered at a dose of 150 mg/d orally until disease progression or intolerable toxicity. Analysis of epidermal growth factor receptor gene mutations in exon 18–21 by direct sequencing was performed in tumor tissue specimens obtained at the first diagnosis.

Results: Sixty-two patients were enrolled and 60 patients were evaluable for efficacy. Objective response rate and disease control rate were 28.3% and 50.0%; median time to progression and overall survival were 77 days and 14.7 months, respectively. In logistic regression analysis, only smoking history was proved to be a statistically significant predictive factor for response (odds ratio: 0.06, $p < 0.001$). Only 7 patients had samples available for mutation analysis. Three patients who had deletion mutations on exon 19 (del E746-A750 or del S752-I759) exhibited objective response. Common toxicities were rash (98%), dry skin (81%), and diarrhea (74%). Discontinuation due to adverse events occurred in 11 patients (18%). Four patients (6%) experienced interstitial lung disease-like events, one of whom died.

Conclusion: Erlotinib is efficacious in Japanese patients with previously treated NSCLC. The toxicity profile was similar to that in Western patients, except for a somewhat higher incidence of skin disorders and interstitial lung disease. Further studies are needed to determine the relationship between epidermal growth factor receptor mutations and outcomes with Erlotinib in Japanese patients.

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Lung cancer affects approximately 1.2 million people annually, and is the leading cause of cancer death in the world.[1] More than 80% of affected patients are diagnosed with non-small cell lung cancer (NSCLC). The standard first-line treatment for metastatic NSCLC is a combination of platinum chemotherapy with a third-generation agent such as docetaxel, paclitaxel, gemcitabine, vinorelbine, and irinotecan.[2,3] Although patients with stage II, IIIA, or IIIB NSCLC receive platinum-based chemotherapy as part of combined modality treatment with thoracic radiotherapy or surgery, many will be candidates for second or third-line chemotherapy. Docetaxel is the only cytotoxic agent with a proven survival advantage over supportive care in patients with disease progression after cisplatin-based chemotherapy for NSCLC.[4] The other agent for which a survival benefit has been demonstrated in this setting is erlotinib,[5] which was approved in Japan for the treatment of relapsed NSCLC in October 2007. Erlotinib is a selective, orally active epidermal growth factor receptor tyrosine kinase inhibitor (EGFR-TKI). In contrast to the experience with the cytotoxic chemotherapeutic agents, response to treatment with EGFR-TKIs has been reported to be influenced by gender, histological type, race or ethnic origin, and smoking status.[5–8]

Tumor molecular markers, including *EGFR* gene mutations and protein expression, have been widely studied in patients with NSCLC, and there is strong evidence that the presence of *EGFR* gene mutations is a predictor of tumor response and resistance.[9–12] However, few prospective studies have evaluated molecular markers as predictors of outcome, and their clinical usefulness is unproven.

This report presents the results of the first phase II study of erlotinib conducted in Japanese patients with NSCLC. The purpose was to evaluate the efficacy and safety of erlotinib in this population. Where available, tumor biopsy samples were analyzed for EFGR-related markers.

PATIENTS AND METHODS

This phase II, multicenter, open-label study recruited patients at 11 hospitals in Japan. The primary end point was the objective response rate (ORR) to erlotinib treatment (150 mg/d). Secondary endpoints were disease control rate (DCR), response duration, time to progression, overall survival (OS), quality of life (QoL), and safety. The protocol was approved by the ethics review boards of all participating institutions, and conducted in accordance with Japanese Good Clinical Practice guidelines.

Patient Selection

Patients with histologically or cytologically documented stage IIIB or IV NSCLC at study entry (not curable with surgery or radiotherapy) that was recurrent or refractory to treatment with one or more chemotherapy regimens (including at least one platinum-containing regimen), were enrolled into this study. Additional eligibility criteria included: the presence of measurable lesions by Response Evaluation Criteria in Solid Tumors (RECIST); age $\geq$20, $<$75 years; Eastern Cooperative Oncology Group performance status (ECOG PS) of 0–2, and adequate bone marrow, hepatic, and renal function, i.e., aspartate aminotransferase and alanine aminotransferase (ALT) levels $\leq$2.5 times the upper limit of normal and total bilirubin of $\leq$1.5 times the upper limit of normal. Patients with existing or previous interstitial lung disease (ILD) were excluded, although a history of radiation pneumonitis (limited to the field of radiation treatment) was permitted. Concomitant anticancer treatment and prophylactic medication for adverse events (AEs) were not permitted, nor was prior use of anti-EGFR or anti human epidermal growth factor receptor (HER2) agents (small molecules and monoclonal antibodies). Written informed consent was obtained from all patients.

Treatment Procedure

After completion of the baseline assessments (see below), all patients received erlotinib (150 mg orally) each morning, 1 hour before breakfast, until the occurrence of progressive disease (PD) or unacceptable toxicity (all AEs were graded using the National Cancer Institute Common Toxicity Criteria Version 2.0). In the event of treatment-related toxicity, 2 dose reductions of 50 mg were permitted per patient, and dosing could also be interrupted for up to 14 days. For grade 3 or intolerable grade 2 rash, treatment was withheld until the rash improved to grade 2 or less, when a lower dose of erlotinib was initiated. For grade 3 diarrhea, treatment was withheld until the diarrhea was grade 1 or less, when a lower dose was started. For ILD of any grade, or any grade 4 toxicity, treatment was immediately and permanently discontinued.

Evaluation of Efficacy

Objective tumor response was assessed in accordance with RECIST.[13] Tumor assessments were performed at baseline, then every 4 weeks until week 16, and then every 8 weeks thereafter. Confirmation of complete or partial responses (PR) was required, by means of a second assessment conducted 28 days or more after the initial assessment. Stable disease (SD) was defined as disease control (absence of progression) maintained for at least 6 weeks. An independent response evaluation committee consisting of 2 oncologists and a radiologist reviewed images of patients with complete response, PR, and SD. Individual survival times were determined from the survival status of each patient during the study period and at the post study follow-up survey conducted in June-July 2005 and May-July 2006. OS was defined as the time from first administrated to death.

Quality of Life Evaluation

The Functional Assessment of Cancer Therapy-Lung (FACT-L) questionnaire (Version 4-A)[14] was used to assess QoL. The full FACT-L questionnaire was administered at baseline and then every 28 days. In addition, the Lung Cancer Subscale (LCS), an independently validated component of FACT-L, was administered weekly during the treatment period. Best responses on the LCS were analyzed for all patients with a baseline LCS score of 24 or less (out of a possible 28 points) and symptomatic improvement was defined as an increase from the baseline score of 2 or more points, sustained for at least 4 weeks.

Evaluation of Safety

Baseline assessment included a full patient history, physical examination, standard laboratory tests, electrocardiography, chest radiography, pregnancy test, and ophthalmologic tests (vision test and slit-lamp examination). Every week until week 8 and every 2 weeks thereafter, vital signs and ECOG PS were monitored and blood samples were taken for hematology and blood chemistry tests. A radiograph examination to assess pulmonary toxicity was conducted weekly until week 4 and every 2 weeks thereafter. Ophthalmologic examinations were repeated at week 8 and at the end of the study. Observation and evaluation of AEs was conducted as appropriate throughout the study period. All AEs were graded using National Cancer Institute Common Toxicity Criteria Version 2.0. For all ILD-like events, the data safety monitoring board (which consisted of oncologists and pneumonologists) reviewed the clinical data and images; the images were also examined by a review committee of radiologists with expertise in drug-induced pulmonary disorders.

Biomarker Analysis

EGFR mutations and EGFR and HER2 protein expression were assessed in patients with suitable tumor tissue specimens at first diagnosis or surgery; these assessments were done only with separate written consent. Tumor samples were obtained from each center as formalin-fixed and paraffin-embedded blocks, or as thinly sliced tissue sections mounted on glass microscope slides. For the mutation analysis, the tissue was microdissected by Targos Molecular Pathology (Kassel, Germany) and direct sequencing was conducted at the Roche Centre of Medical Genomics (Basel, Switzerland), using a nested polymerase chain reaction of exon 18–21. EGFR protein expression was analyzed by Lab Corp (Mechelen, Belgium). EGFR expression analysis was conducted by immunohistochemistry using Dako EGFR PharmDx™ kits (Dako, Carpinteria, CA). A positive test was

defined as membranous staining in ≥10% of the tumor cells. HER2 protein expression was measured using HercepTest™ (Dako, Carpinteria, CA), and a score of 1+ or above (possible scores were: 0, 1+, 2+, 3+) was regarded as positive.

Statistical Analysis

Given an expected ORR of 20%, a Fisher's exact test was performed (one-sided $\alpha = 2.5\%$). Based on 50 patients, the power to test the null hypothesis (ORR = 5%) was 89.66%. The target sample size of 60 patients was chosen on the expectation that a proportion of patients would prove to be ineligible for the study. The main analysis of efficacy was conducted on the full analysis set (FAS), which was produced by omitting ineligible patients. The 95% confidence interval (CI) for ORR, DCR, and symptom improvement rate was calculated by the Clopper-Pearson method. The time-to-event variables were estimated by the Kaplan-Meier method. Logistic regression and Cox proportional hazards regression analysis was conducted on best response and survival time, respectively. In both cases, univariate and multivariate analyses were used to evaluate the effects of 11 factors relating to patient and disease characteristics, and previous treatment.

RESULTS

Patient Characteristics

A total of 62 patients were enrolled between December 2003 and January 2005. All were evaluable for safety and 60 were evaluable for efficacy (FAS). Two patients did not have a measurable lesion according to RECIST. The baseline characteristics of the patients, including their treatment history, are shown in Table 1. The median age was 60.5 years (range: 28–74 years), and 71% of patients were male. Fifty-seven patients (92%) had adenocarcinoma, and 20 (32%) were never-smokers. Twenty-seven patients (44%) had received only one previous chemotherapy regimen.

Efficacy

Tumor response rates in the FAS (as assessed by extrainstitutional review) are shown in Table 2. Seventeen patients were assessed as having a PR and 13 as having SD. The ORR was 28.3% (95% CI: 17.5–41.4%) and the DCR was 50% (95% CI: 36.8–63.2%). In three patients, objective response could not be adequately confirmed, because each discontinued treatment early in the study due to AEs. The median duration of response was 278 days (95% CI: 203–422 days), and time to progression was 77 days (95% CI: 55–166 days). OS was determined based on information collected until the follow-up survey conducted in May–July 2006. The median survival time was 14.72 months (95% CI: 11.07–20.57 months; 19 censored cases) and the 1-year survival rate was 56.5% (95% CI: 43.9–69.1%) (Figure 1). The median OS of patients with PD was 9.95 months. The symptom improvement rate measured using the LCS was 42.1% (24/57; 95% CI: 29.1–55.9%).

The overall response rate was higher in women (58.8%; 10/17) than in men (16.3%; 7/43, χ^2 test: $p = 0.0029$), and in never-smokers (63.2%; 12/19) than in current or former smokers (12.2%; 5/41, $p = 0.0002$). There was no statistically significant difference between the response rate in patients with adenocarcinoma (28.6%; 16/56) and nonadenocarcinoma histology (25.0%; 1/4, $p = 1.0000$). The response

TABLE 1. Summary of Baseline Patient Characteristics and Demographics

Patient and Disease characteristics	No. of Patients ($n = 62$)	%
Age (yr)		
Median	60.5	
Range	28–74	
Sex		
Female	18	29
Male	44	71
Performance status		
0	20	32
1	41	66
2	1	2
Histology		
Adenocarcinoma	57	92
Squamous cell	4	6
Unclassified	1	2
Stage		
IIIB	8	13
IV	54	87
Smoking history		
Never smoked	20	32
Current- or former smoker	42	68
Time since initial diagnosis (d)		
Median	304.0	
Range	2–2353	
Prior chemotherapy regimens		
1	27	44
2	23	37
≥3	12	19
Prior taxanes		
No	10	16
Yes	52	84
Time since last regimen (d)		
Median	80.0	
Range	29–528	

TABLE 2. Response Assessment

Parameter	n	(%)
Partial response	17	28.3
Stable disease	13	21.7
Progressive disease	27	45.0
Not assessable	3	5.0
Response rate (%) (95% CI)	28.3 (17.5–41.4)	
Disease control rate (%) (95% CI)	50.0 (36.8–63.2)	
Duration of response (median: days)[a] (95% CI)	278 (203.0–422.0)	
Time to progression (median: days)[a] (95% CI)	77 (55–166)	

[a] Kaplan–Meier method.
CI, confidence intervals.

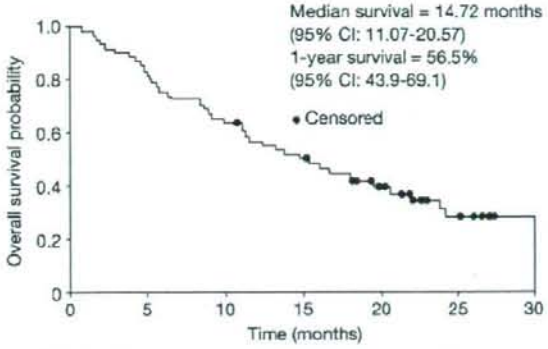

FIGURE 1. Kaplan-Meier plot showing overall survival.

rate was not affected by the number of previous chemotherapy regimens, however, being 27% for patients with one previous regimen (7/26) and 29% for those with 2 or more regimens (10/34). No statistically significant differences were found between other patient subgroups. In a multivariate logistic regression analysis, only smoking history was found to be a statistically significant predictor of response. A multivariate Cox regression analysis showed that both smoking history and ECOG PS were significant predictors for OS (Table 3).

Safety

All 62 patients who received erlotinib were assessed for safety. Treatment-related AEs were observed in all patients, and there were 24 serious AEs in 18 patients (29%). AEs led to discontinuation of erlotinib in 11 patients (18%), including 3 due to ILD-like events, 2 due to ALT elevation, and one each due to rash, paronychia, punctate keratitis, dyspnea/hypoxia, pneumonia and fever/inflammatory neck swelling, and to dose interruptions in 30 patients (48.4%). While the main reasons for the dose interruptions were rash (n = 15; 24.2%) and diarrhea (n = 4; 6.5%), only one patient with rash

TABLE 3. Logistic and Cox Regression Analysis

	Odds Ratio[^]	(95% CI)	p
Logistic regression analysis of response			
Univariate analysis			
Sex (female vs male)	0.14	0.04–0.48	0.002
Age (<65 vs ≥65)	1.26	0.38–4.13	0.704
Histology (non-AD vs AD)	1.20	0.12–12.41	0.878
Smoking history (never vs current or former)	0.08	0.02–0.30	<0.001
Performance status (0 vs ≥1)	0.62	0.19–1.98	0.420
Prior regimens (1 vs ≥2)	1.13	0.36–3.53	0.832
Stage (IIIB vs IV)	0.99	0.17–5.65	0.988
KL-6 (baseline) (<median [496.5 U/ml[a]] vs ≥median)	1.64	0.53–5.12	0.392
Best response to previous chemotherapy (non-PR vs PR)	0.90	0.24–3.33	0.869
Prior taxanes (no vs yes)	0.43	0.10–1.84	0.253
Time since initial diagnosis (≤12 mo vs >12 mo)	1.02	0.31–3.30	0.976
Multivariate analysis			
Smoking history (never vs current or former)	0.06	0.02–0.28	<0.001
Time since initial diagnosis (<12 mo vs ≥12 mo)	2.22	0.49–10.20	0.304
Cox regression analysis of survival			
Univariate analysis			
Sex (female vs male)	1.76	0.85–3.61	0.126
Age (<65 vs ≥65)	0.86	0.44–1.71	0.675
Histology (non-AD vs AD)	0.55	0.19–1.55	0.255
Smoking history (never vs current or former)	1.90	0.93–3.90	0.079
Performance status (0 vs ≥1)	2.31	1.12–4.73	0.023
Prior regimens (1 vs ≥2)	0.93	0.50–1.75	0.833
Stage (IIIB vs IV)	1.38	0.49–3.89	0.542
KL-6 (baseline) (<median [496.5 U/ml[a]] vs ≥median)	1.64	0.87–3.06	0.125
Best response to previous chemotherapy (non-PR vs PR)	0.66	0.31–1.44	0.300
Prior taxanes (no vs yes)	2.09	0.74–5.90	0.163
Time since initial diagnosis (≤12 mo vs >12 mo)	0.76	0.40–1.47	0.418
Multivariate analysis			
Smoking history (never vs current or former)	2.20	1.06–4.56	0.035
Performance status (0 vs ≥1)	2.59	1.25–5.37	0.011

[a] Or 629 ng/ml.
[^] Left site of 'vs' indicates reference group.
PR, partial response; AD, adenocarcinoma; CI, confidence interval.

TABLE 4. Major Treatment-Related Adverse Events and Interstitial Lung Disease-Like Events

			NCI-CTC Grade (*n*)			
Event[a]	*n*	%	1	2	3	>4
Rash	61	98.4	18	41	2	0
Dry skin	50	80.6	44	6	—	—
Diarrhea	46	74.2	33	10	3	0
Pruritus	45	72.6	38	7	0	—
Stomatitis	24	38.7	19	4	1	0
Fatigue	21	33.9	15	6	0	0
Anorexia	19	30.6	11	6	2	0
Paronychia	18	29.0	12	5	1	0
C-reactive protein increased	15	24.2	8	7	0	0
Alanine aminotransferase increased	15	24.2	11	2	2	0
Total bilirubin increased	15	24.2	8	7	0	0
Weight loss	13	21.0	13	0	0	—
ILD-like events	4	6.5	1	0	2	1[b]

Case	Sex	Age	Smoking History	Brinkman Index	Performance Status	Histology	Onset (day)	Outcome	Relation to Erlotinib[c]
1	Male	75	Former	640	1	Adenocarcinoma	52	Recovery	Probable
2	Male	67	Never	—	1	Adenocarcinoma	103	Death (145)	Possible
3	Female	39	Never	—	0	Adenocarcinoma	85	Recovery	Probable
4	Male	69	Former	1000	1	Adenocarcinoma	13	Recovery	Unlikely

[a] Categorized by MedDra Ver.7.1 (except for event).
[b] Grade 5.
[c] Judged by ILD review committee.
NCI-CTC, National Cancer Institute Common Toxicity Criteria; ILD, interstitial lung disease.

had to discontinue treatment, and no patients had to discontinue because of diarrhea or any other digestive toxicity. Fourteen patients (23%) had dose reductions due to AEs, mostly due to rash ($n = 9$; 15%). Treatment-related AEs with an incidence of 20% or more are shown in Table 4; the main events were rash (98%), dry skin (81%), and diarrhea (74%). Elevated laboratory test values related to liver function were found in some patients (total bilirubin: 24%, ALT: 24%), and grade 3 ALT elevation led to treatment discontinuation in 2 patients. Four patients had ILD-like events, including worsening of radiation pneumonitis in one patient, and one died (Table 4). All four (three men; one woman) had an ECOG PS of 0–1 and 2 were former smokers. The patient who died was a 67-year-old man with adenocarcinoma and no history of smoking who discontinued treatment on day 84 due to PD. He developed interstitial pneumonia on day 103 and received 3 days of palliative thoracic irradiation from day 99, after completing the study (3 Gy × 3 days). A computed tomography scan showed characteristic features of ILD (cryptogenic organizing pneumonia-like pattern), and the ILD review committee decided that use of erlotinib could not be excluded as the cause. For the patient with worsening of radiation pneumonitis (case 4), the committee concluded that there was a possible influence of previous radiation therapy, and that this could be seen in the computed tomography scan on day 1. There was, therefore, little reason to suspect that the use of erlotinib had been the cause. Rather, it appeared that the radiation pneumonitis had worsened according to the normal course of illness.

Biomarker Analysis

Tissue samples for measurement of *EGFR* mutations were available for 16 of the 60 patients evaluated for efficacy. For 7 patients, all base sequences were successfully identified in the 4 segments of exons 18–21. All seven (three men, four women) had adenocarcinoma; three were never-smokers, three former smokers and one a current smoker. Three had PR, two SD and two PD. Five of the seven patients had *EGFR* gene mutations and, in all, seven different mutations were detected. The 3 patients with PR all had deletion mutations in exon 19 (del E746-A750 or del S752-I759). One of the 2 patients with PD had no mutations and the other had 2 substitution mutations: L858R in exon 21 and the resistance mutation T790M in exon 20 (Table 5).

Paraffin-embedded tissue samples for immunohistochemistry were available from 12 patients, among whom, 11 had successful determinations of immunohistochemical staining (including 3 patients with PR). Six of the 11 were found to be EGFR-positive and 4 were HER2-positive. However, there were no notable relationships between the EGFR and HER2 expression status and either tumor response or patient characteristics such as sex, histological type or smoking history (data not shown).

DISCUSSION

The present study was conducted on the basis of results from a phase I study of erlotinib in Japanese patients with solid tumors,[15] which showed erlotinib to be well tolerated at

TABLE 5. *EGFR* Mutation Analysis

Response	TTP (d)	Survival (d)	Sex	Histology	Smoking history	Mutation status	Exon	Type of Mutation
PR	222	546	Female	Adenocarcinoma	Never	+	19	del E746–A750
PR	230	811+	Male	Adenocarcinoma	Current	+	19	del S752 –I759 and T751N
PR	278+	911	Female	Adenocarcinoma	Never	+	19	V786M, del E746–A750
SD	224	649+	Male	Adenocarcinoma	Former	+	21	del V834-
SD	77	737	Female	Adenocarcinoma	Former	−	—	—
PD	60	604+	Female	Adenocarcinoma	Never	+	20, 21	L858R, T790M
PD	19	347	Male	Adenocarcinoma	Former	−	—	—

TTP, time to progression; PR, partial response; SD, stable disease; PD, progressive disease.

a dose of 150 mg/d, as well as a phase II study of erlotinib in NSCLC conducted in the United States.[16] In this study, erlotinib achieved an ORR of 28.3%, which was higher than expected, and a DCR of 50%. The response rate was higher than that determined in the above-mentioned phase II study[16] and in keeping with the rate seen in the Japanese subgroup in the phase II study of gefitinib (IDEAL1; 27.5%).[6] Assessment of QoL using the LCS demonstrated a clinically meaningful rate of symptom improvement of 42.1%.

The characteristics of the patients in this study were generally similar to those of NSCLC patients as a whole, in terms of their demographics and disease and treatment history, with the exception of a particularly high proportion of patients with adenocarcinoma (92%). The possibility of enrollment bias on the basis of histological type cannot be ruled out, in part because enrollment coincided with the emergence of reports that the efficacy of EGFR-TKI therapy was greater in patients with adenocarcinoma.[17] However, we also observed one PR and two SDs among three patients with squamous cell carcinoma (FAS population), and our results do not rule out the efficacy of erlotinib in any patient subtype. A multivariate logistic regression analysis showed that smoking status was significantly associated with tumor response, in agreement with previous studies of predictive factors for response to EGFR-TKIs.[5,18,19]

The median survival time with erlotinib was an encouraging 14.7 months. One of the reasons for this long survival may be the high proportion of never-smokers and patients with adenocarcinoma compared with those of other studies, particularly the multinational phase III erlotinib study (BR.21).[5] On the other hand, the presence of *EGFR* gene mutations is currently regarded as an important determinant of treatment response to EGFR-TKIs[20,21] and may be the most important factor in relation to the favorable results seen in the present study. However, it is important to recognize that the potential prognostic effect of mutation status cannot be excluded. The sample size of this and previous trials limits the interpretation of this effect, which will be adequately assessed only by means of appropriately powered trials specifically designed to examine these factors.

Assessment of the presence or absence of *EGFR* gene mutation was possible in only seven patients in the present study. Despite this, the results were consistent with the results of some previous studies. All three of the patients who had a PR (including a male current smoker) had an in-frame deletion in exon 19, which is considered to be the most frequent mutation site in the EGFR-TK domain.[22] One of the 2 patients with PD had a point substitution mutation (L858R) in exon 21, the second most frequent mutation site,[22] and a point mutation (T790M) in exon 20, which is suggested to be involved in tolerance to EGFR-TKI.[12,23,24] It would be valuable to conduct further prospective randomized studies on the association between these markers and survival during treatment with erlotinib in Japanese patients.

Rash and diarrhea were the main AEs reported by patients on erlotinib treatment, as reported in previous studies.[5,15,16] Rash was observed in almost all patients, and was the main reason for treatment interruptions or dose reductions. Although the protocol allowed treatment to be interrupted for grade 3 rash (or intolerable grade 2 rash), grade 3 rash only occurred in 2 patients, leading to discontinuation of treatment in one. Most cases of rash responded to symptomatic treatment and either interruption or dose reduction of erlotinib. Despite suggestions in some reports that the presence of erlotinib-related rash is associated with treatment efficacy and can be used to predict response,[25] no supportive evidence was found in the present study.

The incidence of ILD, which is the most clinically problematic AE associated with erlotinib, tended to be higher than that reported in other clinical studies of erlotinib.[5,26] This is in keeping with this class of agent, and is not unexpected in the Japanese population.

We would recommend that careful screening of patients for ILD risk factors, particularly signs of interstitial pneumonia and pulmonary fibrosis, is done before erlotinib therapy is initiated. Individuals with any previous history of ILD were excluded from this study.

In conclusion, erlotinib (150 mg/d) was shown to have promising antitumor efficacy in Japanese patients with previously treated NSCLC, leading to clinically meaningful improvements in symptoms and an encouraging median survival time. Despite, as expected, a high rate of rash and diarrhea, erlotinib was well tolerated at a dose of 150 mg/d by the majority of patients.

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ORIGINAL REPORT

Phase III Study, V-15-32, of Gefitinib Versus Docetaxel in Previously Treated Japanese Patients With Non–Small-Cell Lung Cancer

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ABSTRACT

Purpose
This phase III study (V-15-32) compared gefitinib (250 mg/d) with docetaxel (60 mg/m^2) in patients (N = 489) with advanced/metastatic non–small-cell lung cancer (NSCLC) who had failed one or two chemotherapy regimens.

Methods
The primary objective was to compare overall survival to demonstrate noninferiority for gefitinib relative to docetaxel. An unadjusted Cox regression model was used for the primary analysis.

Results
Noninferiority in overall survival was not achieved (hazard ratio [HR], 1.12; 95.24% CI, 0.89 to 1.40) according to the predefined criterion (upper CI limit for HR ≤ 1.25); however, no significant difference in overall survival (P = .330) was apparent between treatments. Poststudy, 36% of gefitinib-treated patients received subsequent docetaxel, and 53% of docetaxel-treated patients received subsequent gefitinib. Gefitinib significantly improved objective response rate and quality of life versus docetaxel; progression-free survival, disease control rates, and symptom improvement were similar for the two treatments. Grades 3 to 4 adverse events occurred in 40.6% (gefitinib) and 81.6% (docetaxel) of patients. Incidence of interstitial lung disease was 5.7% (gefitinib) and 2.9% (docetaxel). Four deaths occurred due to adverse events in the gefitinib arm (three deaths as a result of interstitial lung disease, judged to be treatment related; one as a result of pneumonia, not treatment related), and none occurred in the docetaxel arm.

Conclusion
Noninferiority in overall survival between gefitinib and docetaxel was not demonstrated according to predefined criteria; however, there was no statistically significant difference in overall survival. Secondary end points showed similar or superior efficacy for gefitinib compared with docetaxel. Gefitinib remains an effective treatment option for previously treated Japanese patients with NSCLC.

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INTRODUCTION

In Japan, patients with advanced non–small-cell lung cancer (NSCLC) who fail first-line platinum-based therapy often receive second-line docetaxel.[1,2] However, docetaxel has been associated with significant levels of toxicity, especially grades 3 to 4 neutropenia (40% to 67% and 63% to 73% for docetaxel 75 mg/m^2 and 60 mg/m^2, respectively).[1-4] In North America and in European countries, docetaxel,[3,4] pemetrexed,[2] and erlotinib[5] are approved second-line treatments for NSCLC.[3,6]

In phase II trials (IDEAL 1 and 2), the epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor gefitinib (Iressa; AstraZeneca, London, United Kingdom) 250 mg/d showed response rates of 12% to 18% and median survival of 7.0 to 7.6 months in patients who had pretreated advanced NSCLC.[7,8] A subset of Japanese patients in IDEAL 1 demonstrated a higher response rate (27.5%) and longer median survival (13.8 months) compared with the overall population.[9] A phase III study (Iressa Survival Evaluation in Lung Cancer) in patients who had previously treated refractory NSCLC

showed that gefitinib was associated with a nonsignificant trend toward improved overall survival versus placebo.[10] Preplanned subgroup analyses demonstrated a statistically significant increase in survival for gefitinib compared with placebo in patients of Asian origin (hazard ratio [HR], 0.66; 95% CI, 0.48 to 0.91; $P = .010$; median survival, 9.5 *v* 5.5 months) and in never-smokers (HR, 0.67; 95% CI, 0.49 to 0.92; $P = .012$; median survival, 8.9 *v* 6.1 months).[10,11]

Reported here is the first phase III study to compare the effects of targeted therapy (gefitinib) with chemotherapy (docetaxel) on overall survival in Japanese patients with advanced/metastatic (stages IIIB to IV) or recurrent NSCLC who failed one or two chemotherapy regimens.

METHODS

Study Design

This multicenter, randomized, open-label, postmarketing clinical study (V-15-32) compared gefitinib with docetaxel in Japanese patients who had pretreated, locally advanced/metastatic (stages IIIB to IV) or recurrent NSCLC. Patients were randomly assigned by using stratification factors of sex (female *v* male), performance status (PS; 0 to 1 *v* 2), histology (adenocarcinoma *v* others), and study site.

The primary end point was overall survival, and the study aimed to show noninferiority of gefitinib versus docetaxel. Secondary end points were progression-free survival (PFS), time to treatment failure, objective response rate (ORR), disease control rate (DCR), quality of life (QoL), disease-related symptoms, safety, and tolerability.

A late protocol amendment included exploratory end points, such as EGFR gene copy number, protein expression, and mutation status of tumor tissue.

Patients

Patients age 20 years or older were eligible if they had the following: histologically or cytologically confirmed NSCLC (stages IIIB to IV) not amenable to curative surgery or radiotherapy, or postoperative recurrent NSCLC; failure of prior treatment with one or two chemotherapy regimens (≥ 1 platinum-based regimen); life expectancy of 3 months or greater; WHO PS 0 to 2; and measurable disease by Response Evaluation Criteria in Solid Tumors (RECIST). To improve recruitment, the protocol was amended approximately 6 months after study initiation to allow patients without measurable lesions to participate. This was not expected to greatly impact the primary end point.

Treatment

Gefitinib 250 mg/d was administered orally; docetaxel was administered every 3 weeks as a 1-hour intravenous infusion of 60 mg/m^2 (ie, the approved dose in Japan). Patients received treatment until disease progression, intolerable toxicity, or discontinuation for another reason. Poststudy treatment was at physician and patient discretion; a switch to other study treatment was prohibited unless requested by the patient.

Assessments

Overall survival was assessed from date of random assignment to date of death as a result of any cause, or data were censored at the last date the patient was known to be alive. Tumor response by RECIST was performed at baseline, every 4 weeks for the first 24 weeks, and every 8 weeks thereafter. Complete response (CR) or partial response (PR) was confirmed on the basis of two consecutive examinations that were at least 28 days apart. Investigator assessment of best overall tumor response was used for the primary analysis; sensitivity analyses were performed with independent response evaluation committee assessment. PFS was defined as the time from random assignment to the earliest occurrence of disease progression or death from any cause; patients who had not progressed or died at data cutoff were censored at last tumor assessment. QoL was assessed with the FACT-L questionnaire at baseline and every 4 weeks during study treatment until week 12. The FACT-L total score and trial outcome index (TOI; sum of FACT-L physical well-being + functional well-being + additional concerns subscales) were calculated. Disease-related symptoms were assessed weekly with the FACT-L lung cancer subscale (LCS). Improvement was defined as an increase from baseline of at least six points for FACT-L or TOI, or an increase of at least two points for LCS, on two visits that were at least 28 days apart. Adverse events (AEs) were monitored and graded according to the National Cancer Institute Common Toxicity Criteria (NCI-CTC; version 2.0). Routine laboratory assessments were performed. EGFR gene copy number was determined by fluorescent in situ hybridization (FISH).[12] EGFR mutations were assessed by direct sequencing of exon 18 to 21 of chromosome 7. EGFR protein expression was measured by immunohistochemistry with the DAKO EGFR pharmaDxTM kit (DAKO, Glostrup, Denmark).[10]

Statistical Analysis

The primary overall survival analysis was conducted in the intent-to-treat (ITT) population by estimating the HR and two-sided 95.24% CI for gefitinib versus docetaxel, derived from a Cox regression model without covariates (significance level adjusted because of interim analysis). Noninferiority was to be concluded if the upper CI limit was ≤ 1.25. Superiority was concluded if the upper CI limit was less than 1. A total of 296 death events were required for 90% power to demonstrate noninferiority, with the assumption that gefitinib had better overall survival than docetaxel (median survival, 14 *v* 12 months[4]), and the study plan was to recruit 484 patients.

Robustness of the primary conclusion was assessed by supportive analyses in the per-protocol population and by using a Cox regression model with covariate adjustment for sex (male *v* female), PS (0 or 1 *v* 2), tumor type (adenocarcinoma *v* other), smoking history (ever *v* never), number of prior chemotherapy regimens (1 *v* 2), age at random assignment (< 65 years *v* ≥ 65 years), time from diagnosis to random assignment (< 6 *v* 6 to 12 *v* > 12 months), and best response to prior chemotherapy (CR/PR *v* stable disease [SD] *v* progressive disease not assessable/unknown).

Preplanned subgroup analyses were performed on the basis of these covariates. Subgroups were first assessed for evidence of randomized treatment effect by subgroup interactions, to ensure that outcomes between subgroups were likely to be different; then, the subgroups for which evidence existed were examined further.

For PFS, the HR and its 95% CI for gefitinib versus docetaxel were calculated for the population that was assessable for response (defined as patients with ≥ 1 measurable lesion at baseline by RECIST) by using a Cox regression model without covariates. Supportive analyses were performed in the ITT population by using a model adjusted for covariates. Overall survival and PFS were summarized with Kaplan-Meier methods.

The ORR (proportion of CR + PR) and the DCR (proportion of CR + PR + SD $\geq$ 12 weeks) were estimated in the assessable-for-response population and were compared between treatments by generating an odds ratio and a 95% CI from a logistic regression model that included covariates.

The exploratory analysis of biomarker subgroups was performed with similar methods to the overall and clinical subgroup analyses when possible.

RESULTS

Patients

From September 2003 to January 2006, 490 patients were randomly assigned from 50 institutes. In the ITT population, 245 patients were randomly assigned to gefitinib, and 244 patients were randomly assigned to docetaxel; one patient was excluded because of a Good Clinical Practice violation (Fig 1). Treatment groups were generally well balanced for baseline demographics (Table 1), except for some small imbalances in smoking history (7% fewer never-smokers and 10% more ex-smokers in the gefitinib arm). The overall population was representative of an advanced, pretreated NSCLC population in a clinical trial setting in Japan. The median (range) duration of treatment for gefitinib was 58.5 (4 to 742) days and, for docetaxel, was 3 (1 to 12) cycles.

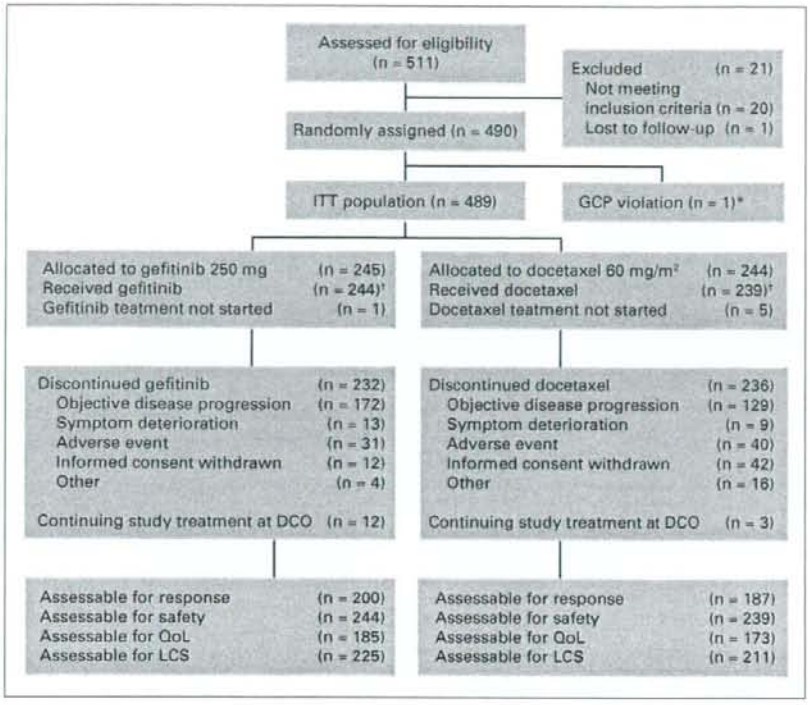

Fig 1. Study flow. (*) Allocated to the docetaxel group. (†) The safety analysis, conducted according to treatment received, was performed on this population. ITT, intent to treat; GCP, Good Clinical Practice; DCO, data cutoff date for overall survival (October 31, 2006); QoL, quality of life; LCS, Lung Cancer Subscale.

Poststudy, 36% of gefitinib-treated patients received subsequent docetaxel, and 40% received no other therapy except for gefitinib; 53% of docetaxel-treated patients received subsequent gefitinib, and 26% received no other therapy except for docetaxel.

Survival

At data cutoff for overall survival (October 31, 2006), overall mortality was 62.6%, and median follow-up was 21 months. Noninferiority in overall survival was not achieved (HR, 1.12; 95.24% CI, 0.89 to 1.40) according to the predefined criterion (upper CI limit for HR $\leq$ 1.25). However, no statistically significant difference in overall survival was apparent (P = .330; Fig 2A).

A supportive Cox analysis, which took into account imbalances in known prognostic factors, showed an HR of 1.01 (95% CI, 0.80 to 1.27; P = .914), which suggested that a demography imbalance that favored docetaxel may have had some impact on the primary, unadjusted, overall survival result.

The median survival and the 1-year survival rates were 11.5 months and 47.8%, respectively, for gefitinib and were 14.0 months and 53.7%, respectively, for docetaxel.

PFS

There was no significant difference between treatments in PFS in the unadjusted analysis (HR, 0.90; 95% CI, 0.72 to 1.12; P = .335); median PFS was 2.0 months with both treatments (Fig 2B). Similar PFS results were obtained from supportive Cox regression analysis adjusted for covariates (HR, 0.81; 95% CI, 0.65 to 1.02; P = .077).

Tumor Response

For ORR, gefitinib was statistically superior to docetaxel (22.5% *v* 12.8%; odds ratio, 2.14; 95% CI, 1.21 to 3.78; P = .009; Table 2). Gefitinib was similar to docetaxel in terms of DCR (34.0% *v* 33.2%; odds ratio, 1.08; 95% CI, 0.69 to 1.68; P = .735). The primary ORR results that were based on investigator judgment were generally consistent with those obtained from independent response evaluation committee assessment.

Symptom Improvement and QoL

Gefitinib showed statistically significant benefits compared with docetaxel in QoL improvement rates (FACT-L: 23.4% *v* 13.9%; P = .023; TOI: 20.5% *v* 8.7%; P = .002; Table 2), but there were no significant differences between treatments in LCS improvement rates (22.7% *v* 20.4%; P = .562).

Subgroup Analyses

Survival outcomes were generally consistent across subgroups, with the exception of best response to prior chemotherapy (treatment by subgroup interaction test P = .017). For patients with best response to prior chemotherapy of progressive disease, overall survival was numerically longer on gefitinib than on docetaxel, whereas patients with a best response of SD had significantly longer survival on docetaxel than on gefitinib (HR, 1.58; 95% CI, 1.09 to 2.27; P = .015; Fig 3A). However, the result was not supported by the PFS (Fig 3B) or ORR results in this subgroup, which favored gefitinib.

Table 1. Baseline Patient Characteristics in Intent-to-Treat Population

Characteristic	Patients per Arm: Gefitinib (n = 245) No.	%	Docetaxel (n = 244) No.	%
Age, years				
≤ 64	138	56.3	135	55.3
≥ 65	107	43.7	109	44.7
Sex				
Male	151	61.6	151	61.9
Female	94	38.4	93	38.1
WHO performance status				
0	85	34.7	93	38.1
1	149	60.8	141	57.8
2	11	4.5	10	4.1
Smoking status				
Ever	174	71.0	157	64.3
Never	71	29.0	87	35.7
Histology				
Adenocarcinoma	192	78.4	188	77.0
Squamous cell carcinoma	37	15.1	41	16.8
Other	16	6.5	15	6.2
Time from diagnosis to random assignment, months				
< 6	70	28.6	60	24.6
6-12	99	40.4	96	39.3
> 12	76	31.0	87	35.7
Disease stage at diagnosis				
IIIB	47	19.2	50	20.5
IV	159	64.9	150	61.5
Recurrent	39	15.9	44	18.0
Number of prior chemotherapy regimens				
1	212	86.5	201	82.4
2	33	13.5	42	17.2
Best response to previous chemotherapy				
CR/PR	113	46.1	106	43.4
SD	91	37.1	101	41.4
PD/NA/unknown	41	16.7	37	15.2
Target lesions at baseline				
Yes	201	82.0	187	76.6
No	44	18.0	57	23.4

Abbreviations: CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; NA, not assessable.

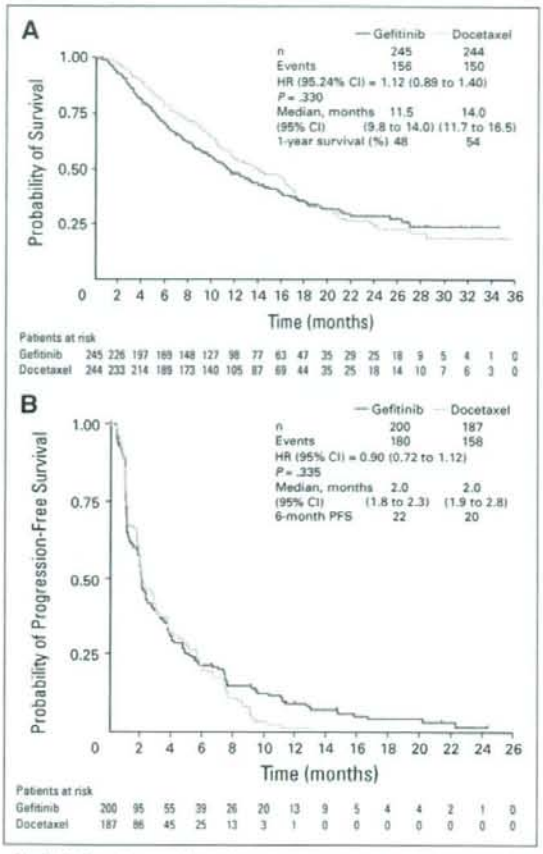

Fig 2. (A) Overall survival in the intent-to-treat population; (B) Progression-free survival (PFS) in the assessable-for-response population. HR, hazard ratio.

Safety

Gefitinib was associated with fewer dose interruptions or delays than docetaxel (26% *v* 52%, respectively). There were no clinically relevant differences in the frequencies of serious AEs or discontinuations of study treatment as a result of AEs between treatment groups (Table 3). Fewer NCI-CTC grades 3 to 4 AEs occurred with gefitinib compared with docetaxel (40.6% *v* 81.6%). There were four deaths as a result of AEs in the gefitinib arm (three as a result of interstitial lung disease that was considered by the investigator to be treatment related; one as a result of pneumonia that was not considered treatment-related), and none in the docetaxel arm.

The most common AEs with gefitinib were rash/acne (76.2%) and diarrhea (51.6%), and the most common AEs with docetaxel were neutropenia (79.5%) and alopecia (59.4%; Table 4). There was a higher incidence of grades 3 to 4 neutropenia with docetaxel (73.6%) compared with gefitinib (8.2%). Interstitial lung disease events occurred in 5.7% (n = 14) and 2.9% (n = 7) of patients who received gefitinib and docetaxel, respectively (Table 3).

Biomarkers

Of the 74 EGFR biomarker samples provided, 53 to 60 were assessable (depending on biomarker). Because of the late protocol amendment, these samples were from long-term survivors who were recruited early or from patients who were recruited later in the study. Compared with the overall study population, this subgroup was over-representative of some stratification factors on both treatment arms: good PS, females, never-smokers, greater than 12 months from diagnosis to random assignment, and best response to prior chemotherapy of CR/PR. There were insufficient events to allow meaningful evaluation of overall survival in relation to biomarker status, and the PFS and ORR data should be interpreted with caution.

Thirty-one (54.4%) of 57 patients had EGFR mutation–positive tumors, and 42 (70.0%) of 60 had EGFR FISH–positive tumors. There

Table 2. Response Rates and Improvement Rates

Rate	Treatment Arm: Gefitinib — Total No. of Assessable Patients	Gefitinib — %	Docetaxel — Total No. of Assessable Patients	Docetaxel — %	Analysis — OR	Analysis — 95% CI	Analysis — P
Response*	200		187				
Overall		22.5		12.8	2.14	1.21 to 3.78	.009
Disease control		34.0		33.2	1.08	0.69 to 1.68	.735
Improvement							
FACT-L	185	23.4	173	13.9	1.89	1.09 to 3.28	.023
TOI	185	20.5	173	8.7	2.72	1.44 to 5.16	.002
LCS	225	22.7	211	20.4	1.15	0.72 to 1.81	.562

Abbreviations: OR, odds ratio; FACT-L, Functional Assessment of Cancer Therapy—Lung (Japanese version 4-A, which includes two additional Japan-specific questions in the subscale on social/family well-being); TOI, trial outcome index; LCS, lung cancer subscale.
*Overall response rate consists of complete response plus partial response rates. Disease control rate consists of the complete response plus partial response rates plus those with stable disease for at least 12 weeks.

was a high degree of overlap between EGFR mutation and clinical characteristics (eg, high frequency in females, in those with adenocarcinoma, and in never-smokers). EGFR mutation–positive patients appeared to have better PFS than EGFR mutation–negative patients on both treatments (gefitinib-positive *v* gefitinib-negative HR, 0.33; 95% CI, 0.11 to 0.97; 17 events; docetaxel HR, 0.15; 95% CI, 0.04 to 0.57; 15 events). In addition, EGFR FISH–positive patients appeared to have better PFS than EGFR FISH–negative patients on both treatments (gefitinib-positive *v* gefitinib-negative HR, 0.75; 95% CI, 0.28 to 1.98; 18 events; docetaxel HR, 0.45; 95% CI, 0.14 to 1.41; 16 events). There were no clear PFS differences between gefitinib and docetaxel in any biomarker subgroups, although the number of events was small and the CIs for the HRs were wide. PFS could not be assessed for EGFR protein expression because of the small number of events in the expression-negative group. For EGFR mutation–positive patients, the ORR was 67% (six of 9 patients) with gefitinib administration and 46% (five of 11 patients) with docetaxel administration. For EGFR FISH–positive patients, the ORR was 46% (five of 11) with gefitinib administration and 33% (six of 18) with docetaxel administration. For EGFR expression–positive patients, the ORR was 36% (five of 14) with gefitinib administration and 31% (four of 13) with docetaxel administration. There were no responses among EGFR mutation–negative, or EGFR FISH–negative, patients, and there was one response (13%) of eight EGFR expression–negative patients who received docetaxel.

DISCUSSION

V-15-32 is the first phase III study to compare gefitinib versus docetaxel in previously treated Japanese patients who have advanced NSCLC. Both gefitinib and docetaxel demonstrated efficacy and tolerability, and findings were consistent with previous experience for both agents in Japan.

Although noninferiority in overall survival for gefitinib versus docetaxel was not proven, there was no statistically significant difference between the two treatments. The original statistical assumption was that gefitinib would have 20% longer survival than docetaxel; hence, the relatively small sample size for a noninferiority study. However, since the study was initiated, data from postmarketing experience in Japan (the SIGN study[13]) and substantial switching to the alternative study treatment on progression in V-15-32 indicated that it would be more likely that gefitinib and docetaxel had similar overall survival. With the assumption of equal survival, the chance (power) of showing noninferiority with this study size is reduced to 48%. The median survival with gefitinib 250 mg/d in our study was consistent with previous experience in Japan (11.5 *v* 13.8 months for Japanese subset of IDEAL 1).[9] Docetaxel demonstrated a longer median survival in V-15-32 (14.0 months) compared with previous Japanese studies (7.8 to 9.4 months).[1,4,14]

In line with increasingly available therapy for NSCLC since the trial was designed and with standard practice in Japan, a large proportion of patients received additional anticancer therapy after discontinuation of the randomly assigned study treatment. Crossover was greater than initially expected, and differences in the number and types of patients who received these poststudy treatments complicated interpretation of survival results. A greater proportion of patients who received docetaxel received poststudy therapy compared with those who received gefitinib. Imbalances in the use of gefitinib after chemotherapy have been reported recently in a phase III study of Japanese patients with lung cancer who were treated with docetaxel and have been cited as a possible explanation for the prolonged median survival seen with docetaxel.[15] INTEREST (Iressa NSCLC Trial Evaluating Response and Survival against Taxotere), a worldwide phase III trial that is comparing gefitinib with docetaxel in pretreated patients who have advanced NSCLC recently demonstrated that gefitinib had statistically noninferior survival to docetaxel.[16] In contrast to V-15-32, INTEREST was larger (1,466 patients) and had subsequent therapies that were well-balanced between treatment arms.

Secondary end points, largely unaffected in this study by subsequent therapy, provided further evidence of the clinical efficacy of both gefitinib and docetaxel in Japanese patients. PFS was similar with gefitinib and docetaxel, and ORR was statistically significantly improved with gefitinib. The ORR in V-15-32 with gefitinib (22.5% *v* 12.8% with docetaxel) was consistent with a subset analysis from IDEAL 1 in Japanese patients (27.5%).[3,8,9]

A number of patient subgroups (including females, patients with adenocarcinoma, and never-smokers) have been reported

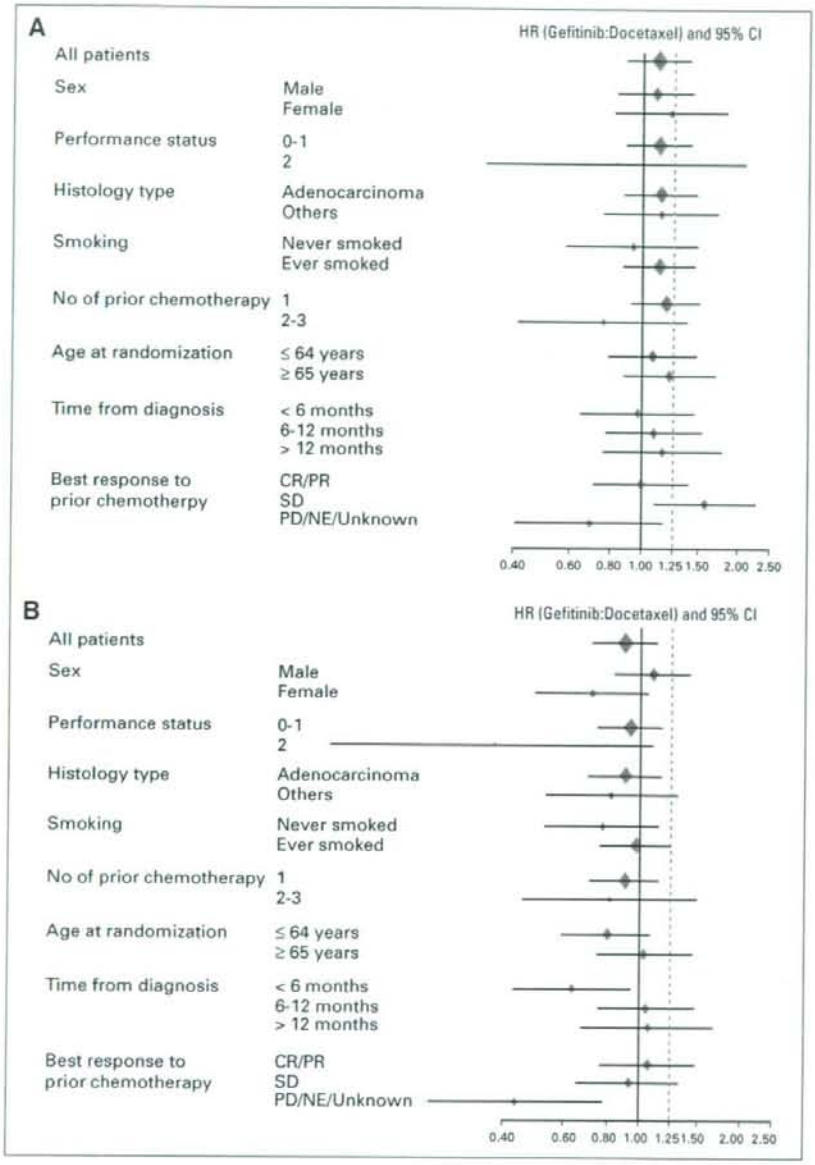

Fig 3. Forest plots of (A) overall survival and (B) progression-free survival that compare treatment groups within clinically relevant subgroups. HR, hazard ratio; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; NE, not assessable.

previously to experience improved clinical benefit with gefitinib.[2,4,7,8,10] Subgroup analyses in this study should be interpreted with caution, as the primary objective was not met, some subgroups were small, and there were imbalances in poststudy treatments. In between-treatment comparisons, no statistically significant overall survival benefit was found for gefitinib compared with docetaxel in any subgroup. However, when post hoc, within-treatment comparisons were performed, females, never-smokers, and patients with adenocarcinoma (and also patients with poor PS and > 12 months since diagnosis) had significantly longer survival than their opposite subgroups on both gefitinib and docetaxel ($P < .001$ for females *v* males, adenocarcinoma *v* others, and never-smokers *v* ever-smokers on both treatments). It appears that the subgroups typically associated with a gefitinib benefit were seen but that they also did well on docetaxel. However, the rate of subsequent gefitinib prescription in the docetaxel arm was high in

Table 3. Summary of Adverse Event Data in the Assessable-for-Safety Population

Category*	Patients			
	Gefitinib (n = 244)		Docetaxel (n = 239)	
	No.	%	No.	%
Adverse events	242	99.2	236	98.7
Treatment-related adverse events	233	95.5	233	97.5
Treatment discontinuation because of an adverse event	33	13.5	42	17.6
NCI-CTC adverse event grades 3 to 4	99	40.6	195	81.6
Serious adverse events	42	17.2	34	14.2
Death as a result of a serious adverse event	4	1.6	0	0
ILD events	14	5.7	7	2.9

Abbreviations: NCI-CTC, National Cancer Institute Common Toxicity Criteria; ILD, interstitial lung disease.
*Participants with multiple events in the same category are counted only once in that category. Participants with events in more than one category are counted once in each of those categories.

these subgroups (eg, approximately two-thirds of docetaxel never-smokers and females had gefitinib as their first poststudy treatment); for PFS and ORR, which are largely unaffected by subsequent treatment, the benefit in these subgroups remained for gefitinib but not for docetaxel, which suggested that poststudy treatments are confounding the interpretation of overall survival in the subgroups.

AEs in our study were consistent with those previously observed, and the most commonly reported AEs were rash/acne and diarrhea for gefitinib and neutropenia for docetaxel. Docetaxel demonstrated a

Table 4. Most Common Adverse Events

Adverse Event	Occurrence by Treatment Arm							
	Gefitinib (n = 244)				Docetaxel (n = 239)			
	Total		Grades 3 to 4		Total		Grades 3 to 4	
	No.	%	No.	%	No.	%	No.	%
Rash/acne*	186	76.2	1	0.4	73	30.5	1	0.4
Diarrhea	126	51.6	5	2.0	67	28.0	2	0.8
Dry skin	90	36.9	0	0.0	13	5.4	0	0.0
Constipation	69	28.3	14	5.7	74	31.0	6	2.5
Anorexia	68	27.9	10	4.1	119	49.8	17	7.1
Nausea	61	25.0	5	2.0	92	38.5	9	3.8
Abnormal hepatic function†	59	24.2	27	11.1	13	5.4	2	0.8
Stomatitis	55	22.5	0	0.0	42	17.6	0	0.0
Nasopharyngitis	50	20.5	0	0.0	32	13.4	0	0.0
Pruritus	42	17.2	0	0.0	15	6.3	0	0.0
Vomiting	41	16.8	4	1.6	41	17.2	3	1.3
Fatigue	36	14.8	1	0.4	107	44.8	6	2.5
Paronychia	33	13.5	1	0.4	2	0.8	0	0.0
Insomnia	32	13.1	0	0.0	20	8.4	0	0.0
Neutropenia‡	24	9.8	20	8.2	190	79.5	176	73.6
Pyrexia	24	9.8	1	0.4	51	21.3	1	0.4
Alopecia	19	7.8	0	0.0	142	59.4	0	0.0
Leukopenia	18	7.4	15	6.1	136	56.9	94	39.3
Headache	12	4.9	1	0.4	25	10.5	0	0.0
Edema§	11	4.5	0	0.0	30	12.6	2	0.8
Myalgia	8	3.3	0	0.0	25	10.5	0	0.0
Dysgeusia	7	2.9	0	0.0	37	15.5	0	0.0
Febrile neutropenia	4	1.6	2	0.8	17	7.1	17	7.1

NOTE. The most common adverse events were considered those that occurred in ≥ 10% of the study population or occurred with > 5% difference between treatments.
*Includes MedDRA high-level terms of rashes, eruptions and exanthems; and of acnes and preferred terms of rash pustular, dermatitis, dermatitis exfoliative, and dermatitis exfoliative generalized.
†Includes MedDRA preferred terms of hepatic function abnormal, alanine aminotransferase increased, aspartate aminotransferase increased and liver disorder.
‡With the exception of one treatment-related adverse event, all other instances of neutropenia reported with gefitinib were in patients who had switched to docetaxel 60 mg/m^2 or other chemotherapy and were reported within the 30-day reporting period. In these other instances, no causal relationship was assigned by the investigator.
§Includes MedDRA preferred terms of edema, edema peripheral, face edema, eyelid edema, and macular edema.

typically high incidence of neutropenia (79.5%) and febrile neutropenia (7.1%) compared with gefitinib (9.8% and 1.6%, respectively). These neutropenia levels that accompanied docetaxel treatment are consistent with previously reported studies in Japanese patients (95.4%[1] and 81.5%[4]). The incidence of interstitial lung disease reported in this study with gefitinib (5.7%) is consistent with that reported in the Japanese postmarketing study (5.8%).[17]

Although the patient numbers were too small for firm conclusions, the biomarker data from this study suggest that EGFR mutation–positive or EGFR FISH–positive patients have a greater response to both gefitinib and docetaxel compared with EGFR mutation– or FISH–negative patients. The gefitinib data are consistent with several previous reports.[18] The docetaxel data provide potential new information about EGFR biomarkers and chemotherapy; this has not been consistently seen before, because there are only a few small studies in the literature, and they have conflicting results.[19] Hence, it is difficult to say conclusively that EGFR mutation or EGFR FISH–positivity predict for docetaxel as well as gefitinib benefit.

Although the study did not prove noninferior survival for gefitinib compared with docetaxel in this patient population, the clinical efficacy and tolerability of gefitinib 250 mg/d in Japanese patients who had NSCLC, reported here, is consistent with the clinical experience reported to date, and gefitinib remains an effective treatment option for previously treated Japanese patients who have locally advanced/metastatic NSCLC.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Although all authors completed the disclosure declaration, the following author(s) indicated a financial or other interest that is relevant to the subject matter under consideration in this article. Certain relationships marked with a "U" are those for which no compensation was received; those relationships marked with a "C" were compensated. For a detailed description of the disclosure categories, or for more information about ASCO's conflict of interest policy, please refer to the Author Disclosure Declaration and the Disclosures of Potential Conflicts of Interest section in Information for Contributors.

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Final approval of manuscript: Yukito Ichinose, Nagahiro Saijo, Masahiro Fukuoka

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