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Tracheo-Esophageal Fistula with Bevacizumab after Mediastinal Radiation

To the Editor:

We report here a case of a young man who developed a trachea-esophageal fistula 4 months following thoracic radiation while being treated with bevacizumab and chemotherapy. A 28-year-old gentleman was diagnosed with non-small cell lung cancer (NSCLC) when he presented with a large right sided mediastinal mass. Transbronchial biopsy results were consistent with adenocarcinoma. Staging evaluation with computerized tomography, flourodeoxyglucose positron emission tomography, and mediastinoscopy confirmed stage IIIB (T2N2M0) disease. He was treated with definitive radiation (74 gray) and concurrent cisplatin with etoposide. One month after completing radiotherapy, he developed progressive disease with enlargement of cervical lymph nodes. Biopsy of a cervical lymph node was consistent with adenocarcinoma. Two months after radiotherapy had been completed, he began systemic treatment with carboplatin, paclitaxel, and bevacizumab (15 mg/kg) every 3 weeks. After two cycles, he had a partial response.

One week prior to his third cycle, he developed progressive odynophagia, then severe coughing with swallowing. An endobronchial evaluation was performed with visualization of a fistulous communication between the esophagus and the trachea, extending into the right mainstem bronchus. An endotracheal stent was placed, but after 2 weeks he had no relief of his respiratory symptoms and was referred to our institution. Bronchoscopy revealed a persistent tracheoesophageal fistula which was not excluded by the endotracheal stent. This endotraheal stent was removed and the fistula was visualized as seen in Figure 1*A*. At that time, a covered esophageal stent (18-mm diameter, 120-mm length, Alveolus) was placed in the esophagus to exclude gastric and oral secretions from the airway (Figure 1*B*). Biopsies of the fistulous tract showed no evidence of malignancy. As the computed tomography scan of the chest and abdomen revealed progressive disease in the mediastinum and liver, an attempt at surgical correction was not considered appropriate. A jejunal feeding tube was placed for nutrition, and he was discharged home with supportive care.

Bevacizumab, a monoclonal antibody targeting vascular endothelial growth factor (VEGF), has been approved for the treatment of advanced NSCLC in combination with paclitaxel and carboplatin.[1,2] Bevacizumab has been associated with bleeding complications, hypertension and gastrointestinal tract perforation.[2] When administered in combination with thoracic radiation, bevacizumab has recently been associated with tracheo-esophageal fistulas. The manufacturer issued a warning based on the development of tracheo-esophageal fistulas in 3 of 29 patients with limited stage small cell lung cancer being treated with definitive radiation, concurrent with irinotecan, carboplatin, and bevacizumab. Data from the manufacturer (as of March 2007) refer to six other instances in which patients with lung and esophageal malignancies developed tracheo-esophageal fistulas while being treated with bevacizumab.[3] A black box warning regarding this complication was mandated by the Food and Drug Administration in April 2007;[2] however, no such reports are available at this time

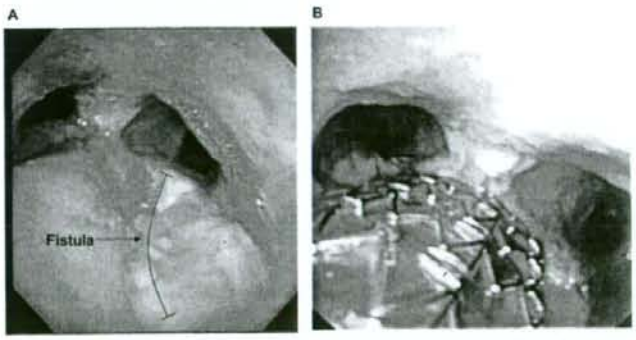

FIGURE 1. *A*, Tracheo-esophageal fistula in patient treated with bevacizumab. *B*, Coated stent in the esophagus, as visualized through the large posterior airway defect.

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Influence of Previous Chemotherapy on the Efficacy of Subsequent Docetaxel Therapy in Advanced Non-small Cell Lung Cancer Patients

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Purpose: To identify factors, particularly the previous use of paclitaxel, that might influence the efficacy of subsequent docetaxel therapy.

Patients and Methods: The patient characteristics, responses, and survivals were compared between the two groups that had received a combination of carboplatin and paclitaxel (group P), and a combination of a platinum and an agent other than paclitaxel (group NP).

Results: A total of 227 patients (127 in group P, and 100 in group NP) were recruited from a hospital-based registry. Two hundred twenty patients were evaluated for the survival, and 210 patients were evaluated for the response of docetaxel therapy. The response rate to docetaxel therapy (14.2% versus 16.0%, $p = 0.702$) or the median survival time (10.9 months versus 11.1 month, $p = 0.567$) did not differ between groups P and NP. The results of multivariate analysis, adjusted for sex, age, and performance status at the start of docetaxel therapy, showed that not the regimen per se, but the response to previous chemotherapy significantly influenced the response rate of docetaxel therapy (odds ratio [OR]: 1.38, 95% confidential interval [CI]: 0.63–3.01; and OR: 2.93, 95% CI: 1.28–6.72, respectively). As for the overall survival, neither the response to nor the previous chemotherapy regimen had any impact (hazard ration [HR]: 0.90, 95% CI 0.66–1.22; HR 0.88, 95% CI 0.65–1.20, respectively).

Conclusion: The previous use of paclitaxel had no impact on the response or survival to subsequent docetaxel therapy. In contrast, the response to previous chemotherapy had a predictive value in relation to responses to subsequent docetaxel therapy in patients with advanced non-small cell lung cancer.

Key Words: Non-small cell lung cancer, Second-line chemotherapy, Docetaxel.

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Lung cancer is a leading cause of cancer-related deaths worldwide.[1] Non-small cell lung cancer (NSCLC) accounts for approximately 80% of all cases of lung cancer. For chemotherapy-naive, patients with advanced NSCLC, with a good performance status (PS), platinum -based chemotherapy has been shown to offer a modest survival benefit over best supportive care alone.[2,3] A high proportion of patients, however, shows disease relapse after initial clinical responses, or progress during the chemotherapy. Thus, a large percentage of patients is moved on to second-line chemotherapy, even though it should only be considered in selected patients with a good PS.[4]

In the landmark study by Shepherd et al., second-line docetaxel thearpy was demonstrated to improve the outcome over best supportive care alone in patients with a history of previous chemotherapy.[5] Since then, a number of agents have been introduced as effective agents for the second-line setting[6–8]; however, the impact of previous chemotherapy on the efficacy of subsequent chemotherapy has not been established.

In relation to small-cell lung cancer, the response of tumors to first-line therapy and recurrence more than 3 months after completion of the initial therapy is often referred to as "sensitive relapse," and absence of tumor response, tumor progression through treatment, or tumor recurrence within 3 months of discontinuation of initial therapy is termed "refractory" disease. Although both are grouped together in most second-line clinical trials, their prognosis and response to salvage therapy have been shown to be different.[9,10] Therefore, in patients with small-cell lung cancer, the efficacy of previous chemotherapy has a significant impact on selection of the subsequent chemotherapy. Whether this relationship between first-and second-line chemotherapy would also apply to cases of NSCLC has not yet been clarified.

In this study, we attempted to identify factors, particularly the previous use of paclitaxel, that might influence the response to subsequent docetaxel therapy in patients with NSCLC. Towards this objective, we divided our patients into two groups according to the previous regimen received.

PATIENTS AND METHODS

We evaluated the patients with histologically or cytologically proven unresectable locally advanced or metastatic

NSCLC, who had received a platinum-containing chemotherapy, and subsequently received docetaxel therapy. The following baseline pretreatment demographic and prognostic information was extracted: age, sex, PS (Eastern Cooperative Oncology Group scale), clinical stage at diagnosis, histology, interval between the final administration of the previous chemotherapy and the start of docetaxel, and response to previous chemotherapy. The platinum-containing therapy was continued for as long as clinical benefit could be observed. Docetaxel was administered at the dose of 60 mg/m^2 and repeated every 3 weeks or longer. We divided these patients into two groups by the initial regimen that they received, namely, combined carboplatin and paclitaxel (group P), or combination of a platinum and an agent other than paclitaxel (group NP).

Objective responses were evaluated using standard bidimensional measurements.[11] Overall survival was measured from the first day of docetaxel treatment until death or the final day of the follow-up period, analyzed using the Kaplan-Meier method, and compared using the log-rank test. Other comparisons were made by χ^2 test, Fisher exact test, and Wilcoxon's test. Factors potentially associated with the efficacy of docetaxel therapy were assessed by univariate and multivariate analysis using the logistic regression model and Cox proportional hazards model. All variables were entered in a single step. Variables tested were sex (male versus female), age (continuous variable), PS at the start of docetaxel therapy (0 versus 1 and 2), regimen of previous chemotherapy (group P versus NP), interval between previous therapy and the start docetaxel chemotherapy (continuous variable), and response to previous chemotherapy (SD/PD versus CR/PR). Differences were considered to be significant at $p < 0.05$. All analyses were performed with Dr. SPSS II (SPSS Japan Inc.).

RESULTS

Patient Characteristics and Docetaxel Delivery

A total of 227 consecutive patients were recruited from a hospital-based registry who were treated with docetaxel after previous platinum-containing chemotherapy between January 2001 and April 2006 at the National Cancer Center Hospital. Of these 127 patients were classified into group P, and 100 into group NP. Seven patients were excluded for the analysis of survival because there was no measurable lesion for the evaluation of response in the previous chemotherapy. Of these 220 patients, another 10 patients were excluded for the analysis of response to docetaxel therapy, because there was no measurable lesion for the evaluation of response in the subsequent docetaxel therapy. By the time of the analysis, 187 out of the 227 patients had died. The median follow-up duration was 10.2 months (range, 0.3–66.9 months) for all patients, and 18.9 months (range, 0.8–66.9 months) for patients who had lost for follow up or alive at the time of analysis.

The patient characteristics are listed in Table 1. The sex and age distributions were similar in the two groups. Stage III disease and a history of previous radiation therapy were slightly predominant in group NP, because concurrent chemoradiotherapy was only administered with the cisplatin (CDDP) and vinorelbine regimen. The response to initial therapy did not differ between the two groups.

TABLE 1. Patient and Disease Characteristics in the Two Groups

	Group P (N = 127)		Group NP (N = 100)		
Characteristics	**No.**	**(%)**	**No.**	**(%)**	p
Sex					
Male	90	(70.9)	79	(79.0)	0.161
Female	37	(29.1)	21	(21.0)	
Age, yr					
Median	58	60		0.072	
Range	30–77		34–75		
Performance status at the start of docetaxel therapy					
0	22	(17.3)	26	(26.0)	0.262
1	101	(79.5)	72	(72.0)	
2	4	(3.2)	2	(2.0)	
Stage at diagnosis					
III	34	(26.8)	51	(51.0)	0.002
IV	72	(56.7)	39	(39.0)	
Recurrence	21	(16.5)	10	(10.0)	
Histology					
Adenocarcinoma	90	(70.9)	68	(68.0)	0.262
Squamous cell carcinoma	23	(18.1)	15	(15.0)	
Large cell carcinoma	2	(1.6)	0	(0)	
Other	12	(9.4)	17	(17.0)	
Interval between the final administration of the previous chemotherapy and the start of docetaxel (wk)					
Median	17		17		0.285
Range	3–134		2–141		
Response to previous chemotherapy					
CR	0	(0)	2	(2.0)	0.031
PR	57	(44.9)	43	(43.0)	
SD	49	(38.6)	46	(46.0)	
PD	17	(13.4)	6	(6.0)	
NE	4	(3.1)	3	(3.0)	
Other treatment					
Radiation	0	(0)	29	(29.0)	<0.001
Surgery	21	(16.5)	10	(10.0)	0.149

CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; NE, not evaluable.

In group NP, the regimens used for the prior chemotherapy and the number of patients treated were as follows; CDDP and vinorelbine (n = 35), combined carboplatin and gemcitabine (n = 24), CDDP and gemcitabine (n = 19), CDDP and irinotecan (n = 18), and others (n = 4).

The median (range) number of cycles of docetaxel chemotherapy administered was 3 (1–17) in group P and 3 (1–13) in group NP.

Efficacy

The response data to docetaxel therapy are summarized in Table 2. There were no significant differences between group P and group NP in terms of the overall response rate (15.1% versus 17.6%), "clinical benefit rate" (79.8% versus 75.6%), or median survival time (6.1 month versus 6.0

TABLE 2. Summary of Docetaxel Therapy in the Two Groups

Characteristics	Group P (N = 127) No.	(%)	Group NP (N = 100) No.	(%)	p
Treatment administration					
Median (range)	3	1–17	3	1–13	0.596
Response to docetaxel therapy					
CR	0	(0)	1	(1.0)	0.256
PR	18	(14.2)	15	(15.0)	
SD	81	(63.8)	54	(54.0)	
PD	24	(18.9)	22	(22.0)	
NE	4	(3.1)	8	(8.0)	
CR/PR	18	(14.2)	16	(16.0)	0.702
CR/PR/SD	99	(78.0)	70	(70.0)	0.173
Median survival time, mo (95% CI)	10.9 (7.6–14.1)		11.1 (8.6–13.5)		0.567

CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; NE, not evaluable.

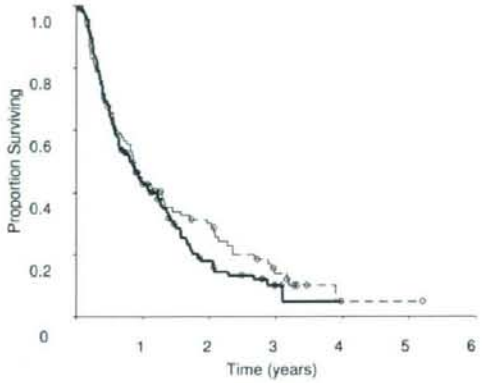

FIGURE 1. Overall survival classified by the previous chemotherapy regimens. Continuous line: carboplatin and paclitaxel (group P, n = 123); and dotted line: platinum and an agent other than paclitaxel (group NP, n = 97). Hazard ratio (95% confidence interval): 1.09 (0.81–1.47).

months) (Figure 1). The response rates to docetaxel in good and poor responders to previous chemotherapy were 21.8% and 9.4%, respectively, in group P (p = 0.074), and 25.0% and 12.0%, respectively, in group NP (p = 0.164). The overall survival did not differ between the good and poor responders (Figure 2).

The result of univariate and multivariate analysis of the response to the docetaxel are shown in Table 3. In the multivariate analysis adjusted for sex, age, PS at the start of docetaxel therapy, the response to previous chemotherapy significantly influenced the response to subsequent docetaxel therapy (odds ratio [OR]: 2.93; 95% CI: 1.28–6.72). The previous chemotherapy regimen (OR: 1.38; 95% CI: 0.63–3.01), and interval between the final administration of the

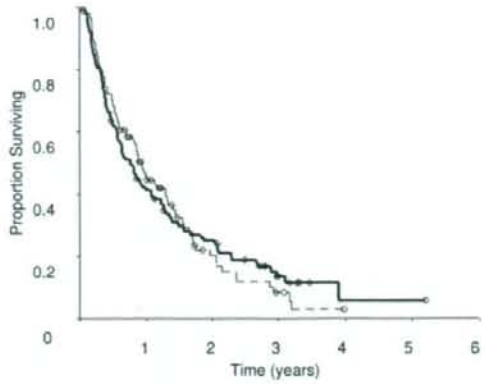

FIGURE 2. Overall survival classified by the responses to previous chemotherapy. Continuous line: SD/PD (n = 118); and dotted line: CR/PR (n = 102). Hazard ratio (95% confidence interval): 0.91 (0.68–1.23).

previous chemotherapy and the start of docetaxel therapy (OR: 0.4; 95% CI: 0.86–1.02) were not found to be significant factors influencing the response to docetaxel therapy. The impact of the responses to the previous chemotherapy was denoted the same tendency in the analysis of each group (OR: 3.82; 95% CI: 1.09–13.5 for group P, and OR: 2.13; 95% CI: 0.67–6.70 for group NP). The result of univariate and multivariate analysis of the overall survival is shown in Table 4. Neither the response to nor the regimen used in the previous chemotherapy had significant impact. Interval between the final administration of the previous chemotherapy and the start of docetaxel therapy were statistically significant in the overall survival.

DISCUSSION

The purpose of this study was to evaluate the influence of previous chemotherapy on the efficacy of subsequent docetaxel chemotherapy. Above all, our major question was whether the regimen of previous chemotherapy, especially the use of paclitaxel, would have any influence on the subsequent docetaxel therapy. In previous studies, response to docetaxel therapy had no association with prior exposure to or the efficacy of paclitaxel therapy, but details about the paclitaxel treatment are not described in these reports.[6,7] In our study, by dividing patients according to the previous regimen received, we showed that the previous use of paclitaxel had no impact on the response to subsequent docetaxel therapy, and that the response to previous chemotherapy was associated with the response to, but not to the survival, after subsequent docetaxel therapy.

Although both paclitaxel and docetaxel are widely used, the influence of prior use of paclitaxel on the response to subsequent docetaxel therapy has not yet been thoroughly reviewed in cases of NSCLC. In the TAX320 study conducted by the Non-Small Cell Lung Cancer Study Group, 31% (114 of 373) of patients had a history of prior use of paclitaxel.[6] In that study, previous exposure to paclitaxel had

TABLE 3. Univariate and Multivariate Analyses of the Response to Docetaxel ($N = 210$)

	Univariate			Multivariate		
	OR	95% CI	*p*	OR	95% CI	*p*
Entire						
Response to previous chemotherapy (SD/PD vs CR/PR)	1.12	0.57–2.50	0.63	2.93	1.28–6.72	0.01
Regimen of previous chemotherapy (group P vs group NP)	0.84	0.40–1.75	0.84	1.38	0.63–3.01	0.421
Interval (with a 30-d increase)	0.97	0.91–1.05	0.48	0.94	0.86–1.02	0.14
Group P						
Response to previous chemotherapy (SD/PD vs CR/PR)	2.70	0.94–7.76	0.07	2.13	0.67–6.70	0.20
Interval (with a 30-d increase)	1.04	0.96–1.12	−0.39	1.01	0.92–1.11	0.06
Group NP						
Response to previous chemotherapy (SD/PD vs CR/PR)	2.37	0.78–7.19	0.13	3.82	1.09–13.5	0.04
Interval (with a 30-d increase)	0.88	0.75–1.02	0.10	0.84	0.69–1.01	0.80

Multivariate analysis was adjusted for sex, age, and performance status at the start of docetaxel.

OR, odds ration; HR, hazard ration; P, carboplatin and paclitaxel; NP, platinum and an agent other than paclitaxel; Interval, days between previous therapy and the start docetaxel chemotherapy; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease.

TABLE 4. Univariate and Multivariate Analyses of Overall Survival ($N = 220$)

	Univariate			Multivariate		
	HR	95% CI	*p*	HR	95% CI	*p*
Entire						
Response to previous chemotherapy (SD/PD vs CR/PR)	0.91	0.68–1.23	0.56	0.90	0.66–1.22	0.484
Regimen of previous chemotherapy (group P vs group NP)	1.09	0.81–1.47	0.57	0.88	0.65–1.20	0.43
Interval (with a 30-d increase)	0.97	0.94–0.99	0.01	0.96	0.94–0.99	0.01
Group P						
Response to previous chemotherapy (SD/PD vs CR/PR)	0.95	0.64–1.41	0.80	0.92	0.60–1.41	0.71
Interval (with a 30-d increase)	0.98	0.94–1.02	0.32	1.01	0.92–1.11	0.13
Group NP						
Response to previous chemotherapy (SD/PD vs CR/PR)	0.86	0.55–1.34	0.86	0.89	0.57–1.40	0.63
Interval (with a 30-d increase)	0.96	0.92–0.99	0.02	0.84	0.69–1.01	0.03

Multivariate analysis was adjusted for sex, age, and performance status at the start of docetaxel.

OR, odds ration; HR, hazard ration; P, carboplatin and paclitaxel; NP, platinum and an agent other than paclitaxel; Interval, days between previous therapy and the start docetaxel chemotherapy; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease.

no impact on the survival of patients who received docetaxel as second-line treatment; however, neither the data of survival nor the details of paclitaxel therapy have been described in the report. In a study comparing pemetrexed and docetaxel in 571 patients, 153 patients (25%) had received paclitaxel.[7] Although the results of the study showed that paclitaxel sensitivity/resistance in the first-line treatment did not predict any difference in the response between pemetrexed and docetaxel used for second-line treatment (details not shown), there were no data comparing the patients according to a history of previous use of paclitaxel.[7] In a study reassessing these data, 20% (113 of 571) of patients had previously received both paclitaxel and platinum, and the previous chemotherapy regimen had no influence on the overall survival.[12] However, the method used for the analysis, namely, assessment of the overall population treated with docetaxel or pemetrexed together, is inappropriate to evaluate the association of previous paclitaxel use with the efficacy of subsequent docetaxel therapy. Patients who had no history of prior taxane treatment were even excluded in some previous phase III studies comparing docetaxel with best supportive care or other agents as second-line treatment.[5,8] In this study, by comparing the patients according to the history of previous use of paclitaxel, we could show specifically that exposure to paclitaxel had no effect on efficacy of subsequent docetaxel therapy.

Although docetaxel and paclitaxel exert their activity via a similar mechanism of action, that is, by interfering with microtubular function and promoting tubulin polymerization and inhibiting the depolymerization of microtubules, the preclinical and clinical activity profiles of the two agents have been shown to exhibit some differences, with partial cross-resistance.[13] Preclinical studies have demonstrated docetaxel to be a 100-fold more potent than paclitaxel in inducing bcl-2 phosphorylation and apoptotic cell death, and the cellular uptake of docetaxel is known to be greater than that of paclitaxel, both of which lead to greater cytotoxic activity of docetaxel.[14] There has been a phase II study of docetaxel in breast cancer patients showing resistance to paclitaxel; objective responses were seen in 18% (8 of 44) of the patients, and the dose or efficacy of previous paclitaxel administration had no impact on the frequency of objective responses. This

indicates that there was perhaps a partial cross-resistance between the two agents in patients of breast cancer.[15] Our study results indicate that this might also be the case in patients of NSCLC.

One of the tentative factors for better survival following second-line chemotherapy is the interval elapsed after the previous chemotherapy. This factor is a possible sign of efficacy of previous chemotherapy, but in the analysis of survival, it is difficult to distinguish whether this factor influences the response to chemotherapy or represents the characteristics of the disease in an individual. Therefore, the interval between two chemotherapy sessions has not been well established as a factor potentially influencing the response in previous studies on NSCLC patients.[5–8,16,17] Some of the studies showed that a longer interval from the last chemotherapy was significantly associated with increased survival.[7,12] In our study, interval between two chemotherapies was associated with the overall survival but not with response, which suggests that this factor have little influence on the antitumor activity of docetaxel therapy, but is representing the characteristics of the tumor.

Difference in the proportions of patients receiving surgery or radiation therapy between the two groups may be a big concern. These local therapies, however, should have only a small influence, if any, because all patients in this study had a metastatic disease at the time of recurrence and start of docetaxel therapy. Although responses to previous chemotherapy in patients treated with chemoradiotherapy could not be evaluated in the same way as the patients treated with chemotherapy alone, the response rates to previous chemotherapy did not differ between the groups P and NP (44.9% in group P, and 45.0% in group NP). Thus, we believe that these populations were appropriately included in our study.

In conclusion, the results of our study showed that docetaxel therapy was similarly active in patients with NSCLC, who had previously been treated with paclitaxel, and the response to previous chemotherapy was predictive of the response to subsequent docetaxel therapy. In the future, many promising agents, whether cytotoxic or molecule-targeted agents, may be developed for the second-line treatment of NSCLC. In the era of abundantly available agents, it will be meaningful to know which patients are likely to derive the most benefit from a particular agent. The results of this study are expected to be helpful for the selection of patients with advanced NSCLC who would benefit from docetaxel therapy.

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Gender Differences in Treatment Outcomes among Patients with Non-Small Cell Lung Cancer Given a Combination of Carboplatin and Paclitaxel

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Key Words
Non-small cell lung cancer · Chemotherapy, carboplatin and paclitaxel

Abstract
Objectives: It was the aim of this study to investigate gender differences in the outcomes of carboplatin and paclitaxel chemotherapy in patients with unresectable stage IIIB-IV non-small cell lung cancer (NSCLC). ***Methods:*** Gender, age, performance status, histology, hematological toxicity, tumor responses and survival parameters obtained retrospectively by medical chart review were analyzed. ***Results:*** A total of 227 patients (147 males and 80 females) were included. The median lowest leukocyte count was 2,900 (range 1,200–12,400)/μl in males and 2,200 (range 600–6,500)/μl in females ($p < 0.001$). Grade 3–4 leukopenia was noted in 15% of male and in 39% of female patients ($p < 0.001$). In both genders, the response rate in evaluable patients was 39%. The median progression-free survival was 4.4 months for men and 5.3 months for women ($p = 0.0081$). After progression of the disease, gefitinib was administered in 64 (44%) male and 45 (56%) female patients, with a median treatment of 35 and 144 days, respectively. The median survival time was 11.9 months for men and 22.2 months for women ($p < 0.001$). ***Conclusion:*** Female gender was associated with a favorable prognosis in patients with NSCLC who received carboplatin and paclitaxel chemotherapy, although the response rates did not differ between the genders. Of note, hematological toxicity was more severe in female patients.

Introduction

Lung cancer remains a major cause of cancer-related death, with an increasing incidence in Japan, as well as world-wide. Non-small cell lung cancer (NSCLC) accounts for more than 80% of lung cancer. Systemic chemotherapy is appropriate for patients with NSCLC if they have extrathoracic metastases or locally advanced disease with a malignant effusion. The standard first-line chemotherapy is a platinum-based doublet regimen, even though it is associated with increased toxicity [1]. Although cisplatin-based regimens are slightly more effective than carboplatin-based regimens, carboplatin is often used due to its more favorable toxicity profile and the fact that it does not require a large intravenous infusion [2]. Among several carboplatin-based regimens, the combination of carboplatin and paclitaxel is frequently used for advanced NSCLC in Japan.

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Lung cancer in women differs from that in men with respect to its incidence, association with smoking and histological distribution [3]. Prospective cohort studies and a population-based study have consistently shown that female gender is a favorable prognostic factor in NSCLC patients; however, these studies included patients of all stages, and their therapy was not specified [4–6]. The presence of a gender difference in survival remains controversial among patients with advanced NSCLC who are treated with systemic chemotherapy; some studies involving multivariate analysis showed better survival in women [7–12], but others showed no difference between men and women [4, 13, 14]. In addition, only a few studies have reported gender differences in tumor responses to chemotherapy [7, 11, 12] and toxicity other than nausea and vomiting [7], which have been reported to be more severe in women [15]. Thus, in the present study, gender differences in survival, tumor responses and toxicity were analyzed in patients with advanced NSCLC who were treated with carboplatin and paclitaxel.

Patients and Methods

Study Population

Patients with unresectable stage IIIB-IV NSCLC who received first-line chemotherapy of carboplatin (AUC = 6, day 1) and paclitaxel (200 mg/m^2, day 1) every 3 weeks at the National Cancer Center Hospital were eligible for this study. A total of 227 patients were identified from January 2001 to July 2005. All patients underwent a systematic pretreatment evaluation and standardized staging procedures. Gender, age, smoking history, performance status, stage, histology, treatment delivery, hematological toxicity, sensory neuropathy, tumor responses and survival parameters were obtained from a retrospective medical chart review. The clinical stage was assigned based on the results of physical examination, chest X-rays, CT scans of the chest and abdomen, CT scans or MRI of the brain and bone scintigrams. The histological classification of the tumor was based on the criteria of the World Health Organization [16]. Toxicity was graded according to the Common Terminology Criteria for Adverse Events version 3.0. Objective tumor responses were evaluated according to the Response Evaluation Criteria in Solid Tumors (RECIST) [17].

Statistical Methods

The demographic, clinical and histopathologic characteristics were compared between the genders. The χ^2 and Mann-Whitney tests were used to evaluate differences in categorical and continuous variables, respectively. Survival curves were calculated according to the Kaplan and Meier method. Cox proportional hazards models were used to adjust potential confounding factors such as smoking history, histology, tumor stage and performance status [18]. All of the above mentioned analyses were performed using the Dr. SPSS II 11.0 for Windows software package (SPSS Japan Inc., Tokyo, Japan).

Table 1. Patient characteristics

Characteristics	Males (n = 147)	Females (n = 80)	p value
Age, years			
Median	61	61	0.60
Range	29–80	27–79	
Smoking history			
All patients			
Smoker	128 (87.1)	22 (27.5)	<0.001
Never-smoker	19 (12.9)	58 (72.5)	
Patients with adenocarcinoma			
Smoker	78 (83.0)	17 (23.9)	<0.001
Never-smoker	16 (17.0)	54 (76.1)	
Patients with non-adenocarcinoma			
Smoker	50 (94.3)	5 (55.6)	0.001
Never-smoker	3 (5.7)	4 (44.4)	
Stage			
IIIB	50 (34.0)	21 (26.3)	0.23
IV	97 (66.0)	59 (73.8)	
Performance status			
0	43 (29.3)	22 (27.5)	0.78
1	104 (70.7)	58 (72.5)	
Histology			
Adenocarcinoma	94 (63.9)	71 (88.8)	<0.001
Squamous cell	27 (18.4)	3 (3.8)	
Others	26 (17.7)	6 (7.5)	

Figures in parentheses are percentages.

Results

Patient Demographics

Of the 227 patients, 147 (65%) were males and 80 (35%) were females (table 1). Smoking history was closely associated with both gender and tumor histology. Eighty-three percent of the male patients with adenocarcinoma had a smoking history compared with only 24% of the female patients. Among patients with non-adenocarcinoma, a gender difference in smoking history was apparent, although the difference was smaller than in adenocarcinoma patients. No significant differences were seen between the genders with respect to age, stage and performance status (table 1).

Chemotherapy Treatment Delivery

The median number of chemotherapy cycles was 3 (range 1–8) in males and 3 (range 1–6) in females (p = 0.21).

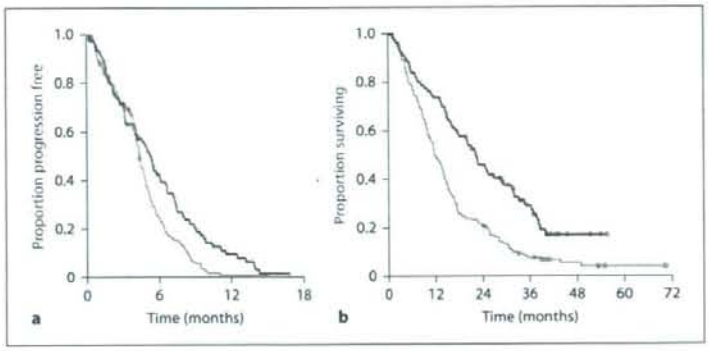

Fig. 1. PFS (**a**) and overall survival (**b**) in all patients. Thick line = Female patients; thin line = male patients.

Table 2. Toxicity

Toxicity	Males (n = 147)	Females (n = 80)	p value
Leukocytopenia			
Median	2,900	2,200	<0.001
Range	1,200–12,400	600–6,500	
Grade 0–2	125 (85.0)	49 (61.3)	<0.001
Grade 3	22 (15.0)	29 (36.3)	
Grade 4	0	2 (2.5)	
Neutropenia			
Median	700	700	0.289
Range	100–11,500	16–3,800	
Grade 0–2	42 (28.6)	20 (25.0)	0.39
Grade 3	56 (38.1)	26 (32.5)	
Grade 4	49 (33.3)	34 (42.5)	
Thrombocytopenia			
Median	13.2	12.4	0.086
Range	2.4–37.3	1.5–34.2	
Grade 0–1	139 (94.6)	73 (91.3)	0.46
Grade 2	7 (4.8)	5 (6.3)	
Grade 3	1 (0.7)	2 (2.5)	
Neurotoxicity			
Grade 0	81 (55.1)	47 (58.8)	0.869
Grade 1	64 (43.5)	32 (40.0)	
Grade 2	2 (1.4)	1 (1.2)	

Figures in parentheses are percentages.

Toxicities

Leukocytopenia during all the chemotherapy cycles was more severe in females than in males (median 2,200/mm^3 vs. 2,900/mm^3, respectively; $p < 0.001$); grade 4 leukocytopenia developed in 39% of females and 15% of males ($p < 0.001$). Grade 4 neutropenia was noted in 43% of females and 33% of males, but this difference was not statistically significant. No gender difference was noted in the frequency of grade 3–4 thrombocytopenia. The severity of neurosensory toxicity was also the same in men and women (table 2).

Response and Treatment after Failure of Initial Chemotherapy

There were 2 complete responses, 52 partial responses, 62 stable diseases and 21 progressive diseases among the 137 male patients evaluable for response, and 1 complete response, 28 partial responses, 33 stable diseases and 12 partial diseases among the 74 female patients evaluable for response; there was no difference in the response rates between male and female patients (39 vs. 39%; $p = 0.999$).

After recurrence or progression of the disease, 64 of the 147 (44%) male patients and 45 of the 80 (56%) female patients received gefitinib monotherapy ($p = 0.067$). The median days of gefitinib treatment was 35 (range 8–803) days in male patients and 144 (range 16–1,325) days in female patients ($p < 0.001$).

Survival

Median progression-free survival (PFS) was longer in females (5.3 months) than in males (4.4 months; $p = 0.0081$) (fig. 1). As of December 2007, 128 deaths had occurred among the male patients and 54 deaths among the female patients. The cause of death was progression of NSCLC, a treatment-related cause, other disease and unknown in 128 (95%), 3 (2.3%), 2 (1.6%) and 2 (1.6%) male and in 50 (93%), 0 (0%), 2 (3.7%) and 2 (3.7%) female patients, respectively. The median survival time (MST) was better in females (22.5 months) than in males (12.5 months; $p < 0.001$). After adjusting for stage, performance status, histology

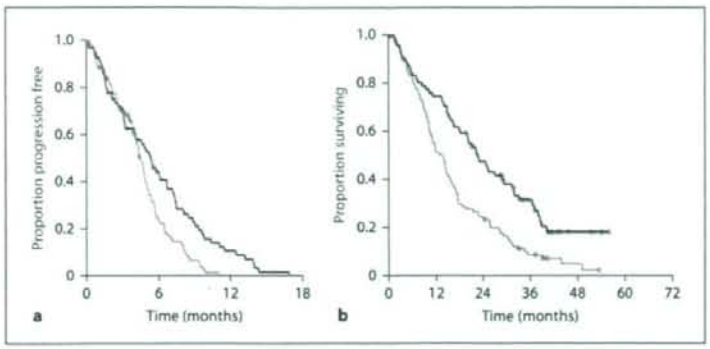

Fig. 2. PFS (**a**) and overall survival (**b**) in patients with adenocarcinoma. Thick line = Female patients; thin line = male patients.

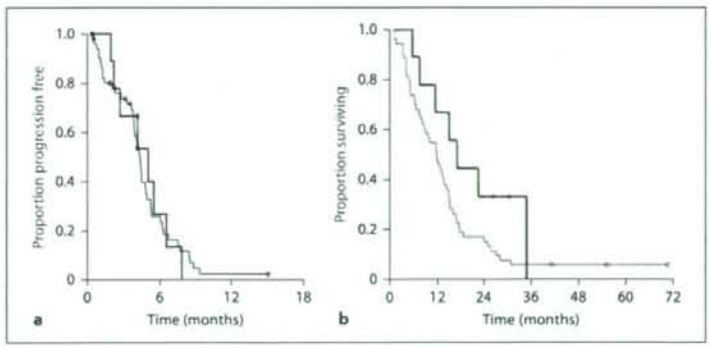

Fig. 3. PFS (**a**) and overall survival (**b**) in patients with non-adenocarcinoma. Thick line = Female patients; thin line = male patients.

and smoking status, female gender was a significant factor for a favorable prognosis (hazard ratio 0.49, 95% confidence interval 0.33–0.73; table 3). In the subset analyses, among patients with adenocarcinoma, PFS and MST were better in females than in males (fig. 2), whereas among patients with non-adenocarcinoma, there was no gender difference in PFS or MST (fig. 3).

Discussion

The present study and other previous studies have shown that female gender is a favorable prognostic factor in patients with stage IIIB or IV NSCLC who receive combination chemotherapy [7–12]. The reasons for this gender difference are currently unknown, but there are 5 possibilities. First, men may not have received sufficient cycles and doses of chemotherapy, since they develop more severe toxicity during chemotherapy than women. How-

Table 3. Multivariate analysis of baseline characteristics for overall survival in all patients

Variables	Patients	Hazard ratio
Sex		
Male	147	1
Female	80	0.49 (0.33–0.73)
Stage		
IIIB	71	1
IV	156	1.37 (1.00–1.89)
Performance status		
0	65	1
1	162	1.31 (0.95–1.81)
Histology		
Adenocarcinoma	165	1
Non-adenocarcinoma	72	1.03 (0.73–1.45)
Smoking		
Never-smoker	77	1
Smoker	150	0.96 (0.65–1.42)

Figures in parentheses are 95% confidence intervals.

ever, in the present study, the number of chemotherapy cycles was the same for both male and female patients, and hematological toxicity was more severe in females than in males. Of note, treatment-related death was observed only in male patients, but the number of deaths was very small (2.7%). The second possibility may be that chemotherapy was more effective in females than in males. However, there was no difference in the response rates by gender in the present study and in previous studies [7, 11, 12]. In 1 study, the duration of response was also found to be the same in male and female patients [11]. The PFS was longer in females than in males in this and in 1 previous study [7], but the PFS can be affected by several factors other than chemotherapy-induced responses. Thus, the second scenario is not likely. The third reason may be that more men die from diseases other than lung cancer. However, in the present study, 95% of male patients and 93% of female patients died of lung cancer progression.

The fourth possibility is that males may have a more aggressive tumor that grows more rapidly than in females. In the present study, there was a higher percentage of never-smokers among female compared with male patients, especially in patients with adenocarcinoma. Large case series studies have found that patients with lung adenocarcinoma who had never smoked had a better survival than those who had a smoking history [19, 20]. Thus, the higher frequency of never-smokers among female patients may explain the better prognosis of female patients in the present study. Recent developments in the molecular pathogenesis of lung cancer suggest that the origins of adenocarcinomas may involve different pathways: a K-RAS mutation-dependent pathway in smokers and an epidermal growth factor receptor mutation-dependent pathway in never-smokers [21]. Lung adenocarcinomas arising by these distinct pathways may have a different potential for progression. Thus, adenocarcinoma in females arising through the epidermal growth factor receptor mutation-dependent pathway may be less aggressive than adenocarcinoma in males, which may arise mainly through the K-RAS mutation-dependent pathway. Carcinogenesis pathways in NSCLC other than adenocarcinoma are unknown, but they are not likely to differ by gender because these tumors are associated with a heavy smoking habit in both genders. These hypotheses are consistent with the results of the present study that there are gender differences in patients with adenocarcinoma, but that the gender differences were small, if any, in those with non-adenocarcinioma.

Finally, gefitinib administration may be associated with a gender difference in overall survival. In the present study, more female patients received gefitinib monotherapy, and the treatment duration was 4 times longer in female than in male patients. Thus, gefitinib treatment probably contributed to the improved survival of female patients.

The present study found that females had more chemotherapy-related hematological toxicity than males during treatment, while there was no gender difference in neurological toxicity. More severe hematological toxicity in females was also noted among patients with SCLC treated with combinations of cyclophosphamide, vincristine, doxorubicin, etoposide and cisplatin [22]. This can be explained by decreased clearance of cyclophosphamide, vincristine, doxorubicin and etoposide due to a 2.4-fold lower expression of hepatic P-glycoprotein, which is a transporter of these agents [23]. The mechanism that could explain the gender difference in toxicity associated with carboplatin and paclitaxel in the present study is unknown, but decreased clearance of paclitaxel is not likely, because neurological toxicity did not differ by gender. Since DNA repair capacity measured using peripheral blood lymphocytes is lower in female lung cancer patients than in male patients [24], increased susceptibility to carboplatin-induced DNA damage may be one factor related to increased chemotherapy-related toxicities in female patients. A recent large-scale study did not show an association between the severity of toxicity and polymorphisms of 16 key genes for drug-metabolizing enzymes, transporters and DNA repair in 914 patients with ovarian cancer who received combination chemotherapy consisting of carboplatin with paclitaxel or docetaxel [25]. However, our understanding of the true regulation of chemotherapy action is very limited at present, and the possibility remains that gender differences in chemotherapy outcome may be based on pharmacogenomic differences between the genders. The lower DNA repair capacity in females may also influence tumor DNA repair after exposure to cytotoxic chemotherapy, and therefore, it may have implications for the significantly longer PFS in female patients after first-line chemotherapy with carboplatin and paclitaxel.

In conclusion, female gender was associated with a favorable prognosis in patients with NSCLC who received combination carboplatin and paclitaxel chemotherapy, even though response rates did not differ by gender. Hematological toxicity was more severe in female patients.

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ORIGINAL REPORT

EGFR Mutations Predict Survival Benefit From Gefitinib in Patients With Advanced Lung Adenocarcinoma: A Historical Comparison of Patients Treated Before and After Gefitinib Approval in Japan

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ABSTRACT

Purpose

This study evaluated whether the presence of *epidermal growth factor receptor* (*EGFR*) mutations is a predictive marker for survival benefit from gefitinib and/or a prognostic marker in patients with advanced lung adenocarcinoma.

Patients and Methods

Overall survival (OS) was compared between patients with advanced lung adenocarcinoma who began first-line systemic therapy before and after gefitinib approval in Japan (January 1999 to July 2001 and July 2002 to December 2004, respectively). Deletional mutations in exon 19 or the L858R mutation in exon 21 of *EGFR* were evaluated using high-resolution melting analysis.

Results

EGFR mutations were detected in 136 (41%) of the 330 patients included in this study. OS was significantly longer among the *EGFR*-mutant patients treated after gefitinib approval compared with the OS of patients treated before gefitinib approval (median survival time [MST], 27.2 *v* 13.6 months, respectively; $P < .001$), whereas no significant survival improvement was observed in patients without *EGFR* mutations (MST, 13.2 *v* 10.4 months, respectively; $P = .13$). A significant interaction between the presence of *EGFR* mutations and a survival improvement was seen ($P = .045$). Among patients treated before gefitinib approval, those with *EGFR* mutations lived longer than those without *EGFR* mutations (MST, 13.6 *v* 10.4 months, respectively; $P = .034$). The response rates to first-line cytotoxic chemotherapy were not significantly different between patients with and without *EGFR* mutations (31% *v* 28%, respectively; $P = .50$).

Conclusion

EGFR mutations significantly predict both a survival benefit from gefitinib and a favorable prognosis in patients with advanced lung adenocarcinoma.

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INTRODUCTION

Gefitinib (Iressa; AstraZeneca, Osaka, Japan) is an orally active, selective epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor (TKI). Gefitinib was approved for the treatment of patients with advanced non–small-cell lung carcinoma (NSCLC) in Japan in July 2002, after its antitumor activity had been demonstrated in two phase II studies.[1,2] The response rate to gefitinib was higher among women, patients with adenocarcinoma, never-smokers, and Japanese or East Asians.[1-3] In April 2004, somatic mutations in the kinase domain of *EGFR*, mainly in-frame deletions including amino acids at codons 747 to 749 (DEL) in exon 19 and a missense mutation at codon 858 (L858R) in exon 21, were suggested to be determinants of gefitinib sensitivity.[4,5] Since then, retrospective studies have consistently revealed a strong association between *EGFR* mutations and clinical outcomes in NSCLC patients treated with gefitinib.[6-9] Although these studies showed that overall survival (OS) was much longer among patients with *EGFR* mutations, they did not intrinsically prove a survival benefit of gefitinib in patients with *EGFR* mutations because there remained the possibility that the differences in OS were merely caused by prognostic differences independent of gefitinib treatment.

Eight large-scale, randomized, phase III trials were conducted to evaluate the survival benefits of

gefitinib or erlotinib (Tarceva; OSI Pharmaceuticals Inc, Melville, NY), another EGFR-TKI, in patients with advanced NSCLC. The Iressa NSCLC Trial Assessing Combination Treatment (INTACT)–1, INTACT-2, Tarceva Responses in Conjunction with Paclitaxel and Carboplatin (TRIBUTE), and Tarceva Lung Cancer Investigation (TALENT) trials tested the concurrent combination of platinum-based chemotherapy and EGFR-TKIs in a first-line setting but failed to show a survival benefit from the addition of the EGFR-TKIs.[10-13] The Iressa Survival Evaluation in Lung Cancer (ISEL) trial tested the role of second- or third-line gefitinib monotherapy but also failed to show a significant survival benefit over a placebo,[14] whereas the BR.21 trial showed a significant survival benefit of second- or third-line erlotinib monotherapy.[15] The Iressa NSCLC Trial Evaluating Response and Survival against Taxotere (INTEREST) and V15-32 trials compared OS after second-line gefitinib monotherapy and docetaxel monotherapy, which is a standard second-line treatment; the former study proved the noninferiority of gefitinib to docetaxel, whereas the latter study failed to do so.[16,17]

In subgroup analyses of some of these trials, significant survival benefits were observed for never-smokers[12,14] and Asian patients.[14] In the BR.21 trial, no history of smoking was a significant predictor of a survival benefit from erlotinib.[15] Because never-smokers and Asian patients are known to have higher frequencies of *EGFR* mutations,[4-9,18,19] these results suggested an association between *EGFR* mutations and a survival benefit from EGFR-TKIs. However, in all of these trials, mutational analyses failed to show a significant survival benefit from EGFR-TKIs in *EGFR*-mutant patients,[20-23] partly because of the small sample sizes that were used.

In the INTACT and TRIBUTE trials, patients with *EGFR* mutations lived longer than those without *EGFR* mutations, irrespective of treatment with EGFR-TKIs[20,21]; this result suggested that *EGFR* mutations may have prognostic value in patients with advanced NSCLC who were treated with standard chemotherapy. However, these trials were inconclusive regarding this point because of the small number of *EGFR*-mutant patients who were examined. As for early-stage NSCLC patients, several large-scale retrospective studies have been reported; some studies showed no significant association between the presence of *EGFR* mutations and OS after surgery,[19,24] whereas others showed that the presence of *EGFR* mutations was associated with a favorable prognosis in a univariate analyses, but the association disappeared when adjustments for patient characteristics like sex and smoking history were made.[25,26]

To evaluate whether gefitinib provides a survival benefit to patients with lung adenocarcinoma and whether the mutational status of *EGFR* is a predictor of a survival benefit from gefitinib and/or a prognostic factor, we analyzed data obtained on patients with advanced lung adenocarcinoma who were treated before and after gefitinib approval.

PATIENTS AND METHODS

Patients

We performed all the analyses in this study using a protocol approved by the institutional review board of the National Cancer Center Hospital (NCCH; Tokyo, Japan). Consecutive patients with advanced lung adenocarcinoma who had been pathologically diagnosed at NCCH and began first-line systemic therapy without thoracic radiotherapy between July 2002 and December 2004 (after gefitinib approval; group A) or between January 1999 and July 2001 (at least 1 year before gefitinib approval; group B) were identified using the databases of NCCH. Patients for whom appropriate pathologic samples were available and a mutational analysis could be successfully performed were included in this study.

Mutational Analysis

DNA was extracted from archived paraffin-embedded tissues and/or Papanicolaou-stained cytologic slides, and the two major hotspots of *EGFR* mutations, DEL and L858R, were analyzed using high-resolution melting analysis according to a previously described method.[9,27] Briefly, polymerase chain reaction (PCR) was performed using primers designed to amplify a region containing E746-I759 or L858 of *EGFR* and the dye LCGreen I (Roche Diagnostics, Indianapolis, IN). Melting curves were obtained using HR-1 (Idaho Technology, Salt Lake City, UT), and the curves of the samples and controls were compared. All of the mutational analyses were performed in a blinded fashion.

Clinical Outcomes

OS was defined as the time from the start of first-line systemic therapy until death. In patients with measurable lesions, tumor response to first-line cytotoxic chemotherapy, including second-line therapy after first-line gefitinib therapy, was evaluated using standard bidimensional measurements.[28] The response rate was defined as the proportion of complete and partial responses compared with the total number of patients.

Statistical Analysis

The differences in OS for the patients in group A and those in group B were compared using Kaplan-Meier curves and log-rank tests. To assess the interaction between the groups and the mutational status of *EGFR*, interaction terms were included in the Cox proportional hazards models. The interaction was considered significant if $P < .10$. The impact of *EGFR* mutations on tumor response to chemotherapy and prognosis was assessed using a χ^2 test and a log-rank test, respectively. These analyses were performed with or without adjustments for the following baseline characteristics: age, sex, smoking history (never-smokers *v* others), performance status (PS), and disease stage (recurrence after surgery *v* stage III/IV). All the statistical analyses were performed using SAS software, version 9.1 (SAS Institute, Cary, NC).

RESULTS

Mutational Analysis

Medical and pathologic records were reviewed for 414 clinically eligible patients (255 in group A and 159 in group B), and the mutational status was successfully determined in 330 patients (200 in group A and 130 in group B). Appropriate pathologic samples were not available in 68 patients (49 in group A and 19 in group B), and indeterminate results were obtained because of incomplete PCR in 16 patients (six in group A and 10 in group B). Of the 330 successfully analyzed patients, 193 were analyzed using only cytology samples, 106 were analyzed using only tissue samples, and 31 were analyzed using both samples. DEL and L858R mutations were detected in 77 (23%) and 59 patients (18%), respectively, and these mutations were mutually exclusive.

Patient Characteristics

The patient characteristics of the 330 patients are listed in Table 1. All of the patients were Japanese except for one Korean patient and one Chinese patient. When groups A and B were compared, group A had a significantly higher percentage of patients with recurrence after surgery and patients with a poor PS. Age, sex, and smoking history were similar between the two groups. In group A, most of the patients were treated with EGFR-TKIs. However, 15 patients (8%) were not treated with EGFR-TKIs, and in 12 patients (6%), the EGFR-TKI

Table 1. Patient Characteristics

Characteristic	Group A: July 2002 to December 2004 (n = 200)		Group B: January 1999 to July 2001 (n = 130)		*P*
	No. of Patients	%	No. of Patients	%	
Age, years					.47
Median	62		62		
Range	27-84		37-84		
Sex					.52
Female	84	42	50	38	
Male	116	58	80	62	
Smoking history*					.70†
Never-smoker	92	46	57	44	
Former smoker	42	21	33	25	
Current smoker	66	33	40	31	
Histologic diagnosis					—
Adenocarcinoma	200	100	130	100	
Other	0	0	0	0	
Performance status					.049‡
0	70	35	46	35	
1	113	57	80	62	
2	13	7	4	3	
3	4	3	0	0	
Stage					.001§
IIIB	37	19	29	22	
IV	79	40	70	54	
Recurrence after surgery	84	42	31	24	
First-line cytotoxic chemotherapy‖					—
Platinum + third-generation drug¶	140	70	88	68	
Other platinum-based regimen	0	0	8	6	
Non–platinum-based regimen	14	7	34	26	
No cytotoxic chemotherapy	46	23	0	0	
EGFR-TKI therapy					—
First line	81	41	0	0	
Second line	63	32	9	7	
Third or more line	29	15	10	8	
Never	15	8	111	85	
Unknown	12	6	0	0	
EGFR mutation status					
DEL	46	23	31	24	
L858R	32	16	27	21	
Wild type	122	61	72	55	

Abbreviations: EGFR, epidermal growth factor receptor; TKI, tyrosine kinase inhibitor; DEL, deletional mutations in exon 19.
*Never-smokers were defined as patients who had never had a smoking habit, and former smokers were defined as patients who had stopped smoking at least 1 year before diagnosis.
†Never-smokers *v* others.
‡0 or 1 *v* 2 or 3.
§IIIB or IV *v* recurrence after surgery.
‖Including second-line therapy after first-line gefitinib therapy.
¶Third-generation drug indicates paclitaxel, docetaxel, gemcitabine, vinorelbine, or irinotecan.

treatment history was unknown because the patients had been transferred to another hospital and the subsequent treatment data was not available. In group B, all but 19 patients (15%) had no history of EGFR-TKI treatment; six patients had been treated with gefitinib in clinical trials before gefitinib approval, one patient had been treated with erlotinib in a phase II trial, and 12 patients had been treated with gefitinib in a clinical practice setting after gefitinib approval.

Historical Comparison Before and After Gefitinib Approval

The median follow-up time for 46 survivors in group A was 30.8 months (range, 10.7 to 49.8 months), and the follow-up times for two survivors in group B were 65.7 and 85.0 months. OS was significantly longer in group A than in group B (median survival time [MST], 18.1 *v* 12.5 months, respectively; hazard ratio [HR] = 0.66; 95% CI, 0.52 to 0.84; $P < .001$; Fig 1A). In group A versus group B, a significant improvement in survival was observed in patients with *EGFR* mutations (MST, 27.2 *v* 13.6 months, respectively; HR = 0.48; 95% CI, 0.32 to 0.71; $P < .001$; Fig 1B), whereas no significant improvement in survival was observed in patients without *EGFR* mutations (MST, 13.2 *v* 10.4 months, respectively; HR = 0.79; 95% CI, 0.59 to 1.07; $P = .13$; Fig 1C). The improvement in survival was similar among patients with DEL (Fig 1D) and those with L858R (Fig 1E). A significant interaction between the mutational status of *EGFR*

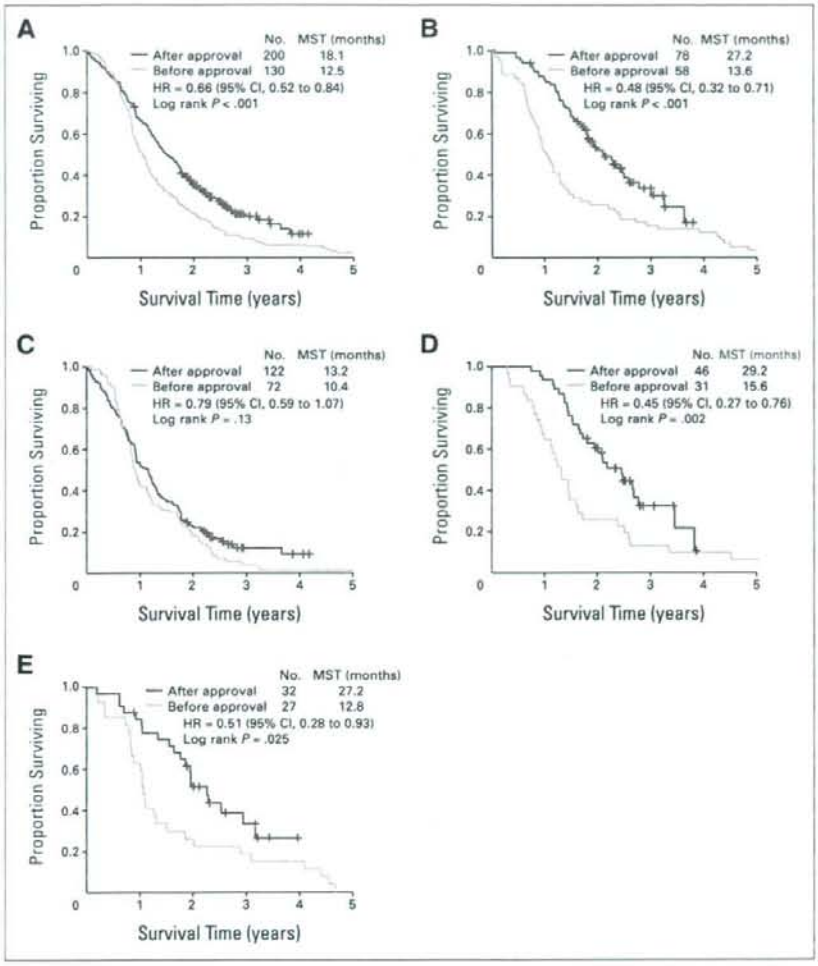

Fig 1. Comparison of overall survival between patients who began first-line systemic therapy after gefitinib approval and patients who began treatment before gefitinib approval. (A) All patients included in the current study. (B) Patients with epidermal growth factor receptor (*EGFR*) mutations. (C) Patients without *EGFR* mutations. (D) Patients with deletional mutations in exon 19. (E) Patients with L858R mutation. MST, median survival time; HR, hazard ratio.

(mutant *v* wild type) and the improvement in survival was observed ($P = .045$). After adjusting for age, sex, smoking history, PS, and disease stage, the HR of after to before gefitinib approval was 0.47 (95% CI, 0.31 to 0.70; $P < .001$) among patients with *EGFR* mutations and 0.76 (95% CI, 0.55 to 1.04; $P = .088$) among patients without *EGFR* mutations. The interaction was also significant after the adjustment ($P = .035$).

Prognosis in Patients Before Gefitinib Approval

When patients with and without *EGFR* mutations were compared in group B (patients treated before gefitinib approval), the patients with *EGFR* mutations lived significantly longer than patients without *EGFR* mutations (MST, 13.6 *v* 10.4 months, respectively; HR = 0.68; 95% CI, 0.48 to 0.97; $P = .034$; Fig 2A), and this finding persisted after adjustments for age, sex, smoking history, PS, and disease stage (HR = 0.65; 95% CI, 0.44 to 0.96; $P = .028$). However, this result may be affected by EGFR-TKI treatment administered to 19 patients (12 with *EGFR* mutations and seven without *EGFR* mutations). When the start of EGFR-TKI administration in the 19 patients was treated as a censoring event to exclude the effect, the difference in OS was not significant (HR = 0.74; 95% CI, 0.50 to 1.08; $P = .12$; Fig 2B). Between patients with DEL and those with L858R, the difference in OS was not significant (MST, 15.6 *v* 12.8 months, respectively; HR = 0.86; 95% CI, 0.51 to 1.46: $P = .58$).

Response to Cytotoxic Chemotherapy

The response to cytotoxic chemotherapy was evaluated in 279 of the 330 patients. The other 51 patients were excluded because no chemotherapy other than gefitinib was administered (n = 46) or they had no measurable lesions (n = 5). As shown in Table 2, the total response rate was 29%, and the response rates were not significantly different between patients with and without *EGFR* mutations (31% *v* 28%, respectively; $P = .50$). These findings were similar for patients with DEL and with L858R (29% *v* 35%, respectively; $P = .49$). *EGFR*

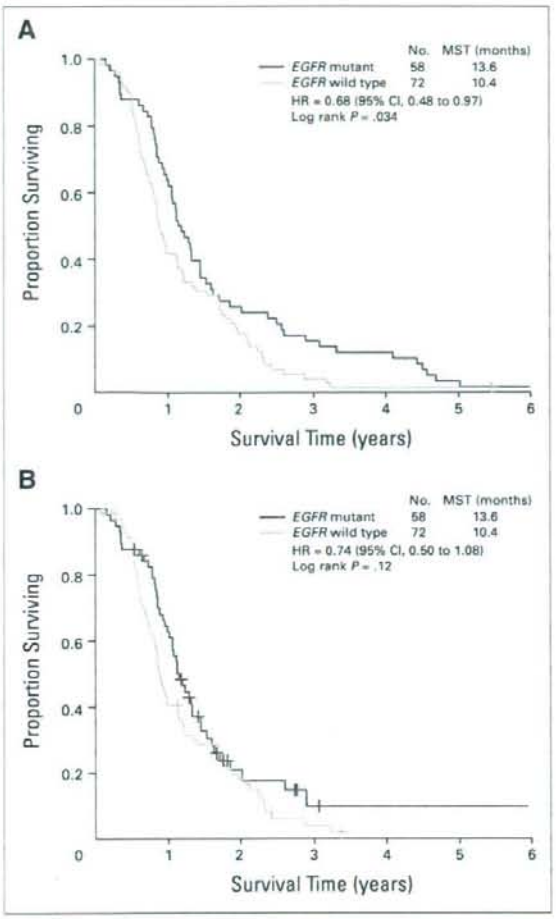

Fig 2. (A) Comparison of overall survival between patients with and without epidermal growth factor receptor (*EGFR*) mutations among patients treated before gefitinib approval; and (B) the same comparison when the start of EGFR tyrosine kinase inhibitor administration is treated as a censoring event. MST, median survival time; HR, hazard ratio.

mutations were not significantly associated with response to any specific regimen, although the response rate to taxane monotherapy tended to be higher among patients with *EGFR* mutations than in patients without *EGFR* mutations (31% *v* 13%, respectively; $P = .17$).

DISCUSSION

To assess the survival benefit of gefitinib in patients with lung adenocarcinoma, we compared the OS of patients treated after gefitinib approval (group A) with a historical control (group B). As the historical control, we selected patients treated between January 1999 and July 2001 because most of these patients routinely received a combination of platinum and a third-generation drug and were also administered second-line cytotoxic chemotherapy, if indicated; thus, their cytotoxic chemotherapy regimens were similar to those of the patients in group A. Actually, fewer cytotoxic chemotherapy regimens were used in group A because some cytotoxic chemotherapy options were replaced with gefitinib therapy. Because the most essential difference between the two groups was the availability of gefitinib, the survival improvement observed in this historical comparison can be interpreted as reflecting a survival benefit from the addition of gefitinib monotherapy or the replacement of cytotoxic chemotherapy with gefitinib monotherapy. Although there was a small number of patients who were not treated with EGFR-TKIs in group A or who were treated with EGFR-TKIs in group B, we included all consecutive patients in the analysis to avoid biases. Some imbalances in the baseline patient characteristics of the two groups were noted; however, all of the results described in the present study were similar even after adjustments were made for the baseline patient characteristics.

In this study, we clearly showed an improvement in the survival of patients with *EGFR* mutations after gefitinib approval. In fact, the MST doubled (13.6 to 27.2 months), a feat that has never before been achieved in the history of NSCLC treatment. Even in patients without *EGFR* mutations, a nonsignificant improvement in survival was obtained (MST, 10.4 to 13.2 months); this result might be a result of the efficacy of gefitinib, period effects other than the approval of gefitinib therapy, or selection biases. Nevertheless, a significant interaction between the presence of *EGFR* mutations and an improvement in survival was obtained, meaning that the mutational status of *EGFR* is a predictor of a survival benefit from gefitinib.

To our knowledge, this is the first study to show a significant interaction between *EGFR* mutations and a survival benefit from EGFR-TKI therapy. Although this study was a retrospective historical comparison conducted only in East Asian patients and some biases could not be excluded, the number of patients with *EGFR* mutations analyzed in this study (n = 136) was much larger than those in phase III trials (INTACT, n = 32; TRIBUTE, n = 29; ISEL, n = 26; BR.21, n = 34),[20-22,29] and we believe that the results of this study have a certain amount of importance to clinical practice.

The current study also showed that, among the patients treated with chemotherapy before gefitinib approval (group B), the OS was significantly longer in the patients with *EGFR* mutations than in those without *EGFR* mutations. As with the INTACT and TRIBUTE trials,[20,21] this result suggested that the presence of *EGFR* mutations was a favorable prognostic factor in patients with advanced NSCLC. However, this result is not conclusive because the difference was marginal when the effects of EGFR-TKIs, which were used in a small number of patients, were excluded.

As for the patients who were treated after gefitinib approval (group A), the difference in OS between the patients with and without *EGFR* mutations can be partly explained by the prognostic value of the *EGFR* mutations themselves. However, this study indicated that the difference was mainly caused by the mutations' predictive value for a survival benefit from gefitinib.

The difference in OS according to the mutational status of *EGFR* in group B can also be explained by the predictive value for chemotherapy efficacy other than the pure prognostic value. In INTEREST and V15-32, which were phase III trials comparing docetaxel and gefitinib, the HRs for OS were almost the same between patients with and without *EGFR* mutations,[16,30] suggesting that *EGFR* mutations might be a predictive factor for a survival benefit from both docetaxel

Table 2. *EGFR* Mutations and Tumor Response to Cytotoxic Chemotherapy

Therapy	Mutant *EGFR*		Wild-Type *EGFR*		*P*	Total	
	No. of Patients	Response Rate (%)	No. of Patients	Response Rate (%)		No. of Patients	Response Rate (%)
Total	112	31	167	28	.50	279	29
Regimens							
Platinum + taxane	54	37	97	34	.71	151	35
Platinum + other third-generation drug*	35	26	39	26	.99	74	26
Taxane† monotherapy	16	31	23	13	.17	39	21
Other regimen	7	14	8	0	.27	15	7
Treatment line							
First line	95	33	147	27	.37	242	29
Second-line therapy after first-line gefitinib therapy	17	24	20	30	.66	37	27

Abbreviation: *EGFR*, epidermal growth factor receptor.
*Other third-generation drug indicates gemcitabine, vinorelbine, or irinotecan.
†Taxane indicates paclitaxel or docetaxel.

and gefitinib. In the current study, response rate to taxane monotherapy tended to be higher in patients with *EGFR* mutations, although the number of patients was small. These results are inconclusive, and further investigation is needed.

We detected no significant difference in the predictive and prognostic values of DEL and L858R in the current study. Some researchers, including ourselves, have reported that patients with DEL had better outcomes after EGFR-TKI treatment than those with L858R[9,31,32]; however, the current study showed that gefitinib yielded almost the same survival benefit to both patients with DEL and patients with L858R, and we think that the two *EGFR* mutations should be treated equally when making clinical decisions.

In the ISEL and BR.21 trials, the *EGFR* copy number (evaluated using fluorescence in situ hybridization), rather than the *EGFR* mutation status, was suggested to predict a survival benefit from EGFR-TKIs,[22,23,29] and the authors concluded that a mutational analysis was not necessary to select patients for treatment with EGFR-TKIs. In contrast, the current study indicated that the *EGFR* mutation status was a determinant of a survival benefit from gefitinib, although *EGFR* copy numbers were not evaluated in this study. Our previous study showed that the *EGFR* copy number, as evaluated using quantitative PCR, was associated with a response to gefitinib; however, an increased *EGFR* copy number tended to be seen in patients with *EGFR* mutations and was not an independent predictor of response or OS in gefitinib-treated patients.[6] These discrepancies may be a result of the ethnic difference, the methodologic difference between fluorescence in situ hybridization and quantitative PCR, or the accuracy of biomarker analyses. Although controversy still remains, we believe that the *EGFR* mutation status is the most useful biomarker for patient selection, at least in East Asian patients who have *EGFR* mutations more frequently than non-Asian patients.

In conclusion, gefitinib yielded a survival benefit among Japanese patients with lung adenocarcinoma, and the survival benefit was significantly greater in patients with *EGFR* mutations than in those without *EGFR* mutations. The presence of *EGFR* mutations may also be a favorable prognostic factor in advanced lung adenocarcinoma independent of gefitinib treatment. We need to consider appropriate treatment strategies for patients with NSCLC based on their *EGFR* mutation status.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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Enhancement of the antitumor activity of ionising radiation by nimotuzumab, a humanised monoclonal antibody to the epidermal growth factor receptor, in non-small cell lung cancer cell lines of differing epidermal growth factor receptor status

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The expression and activity of the epidermal growth factor receptor (EGFR) are determinants of radiosensitivity in several tumour types, including non-small cell lung cancer (NSCLC). However, little is known of whether genetic alterations of *EGFR* in NSCLC cells affect the therapeutic response to monoclonal antibodies (mAbs) to EGFR in combination with radiation. We examined the effects of nimotuzumab, a humanised mAb to EGFR, in combination with ionising radiation on human NSCLC cell lines of differing *EGFR* status. Flow cytometry revealed that H292 and Ma-1 cells expressed high and moderate levels of EGFR on the cell surface, respectively, whereas H460, H1299, and H1975 cells showed a low level of surface EGFR expression. Immunoblot analysis revealed that EGFR phosphorylation was inhibited by nimotuzumab in H292 and Ma-1 cells but not in H460, H1299, or H1975 cells. Nimotuzumab augmented the cytotoxic effect of radiation in H292 and Ma-1 cells in a clonogenic assay *in vitro*, with a dose enhancement factor of 1.5 and 1.3, respectively. It also enhanced the antitumor effect of radiation on H292 and Ma-1 cell xenografts in nude mice, with an enhancement factor of 1.3 and 4.0, respectively. Nimotuzumab did not affect the radioresponse of H460 cells *in vitro* or *in vivo*. Nimotuzumab enhanced the antitumor efficacy of radiation in certain human NSCLC cell lines *in vitro* and *in vivo*. This effect may be related to the level of EGFR expression on the cell surface rather than to *EGFR* mutation.
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Epidermal growth factor receptor (EGFR) is a receptor tyrosine kinase that is abnormally upregulated and activated in a variety of tumours (Baselga, 2002). Deregulation of receptor tyrosine kinases as a result of overexpression or activating mutations is frequently associated with human cancers and leads to the promotion of cell proliferation or migration, inhibition of cell death, or the induction of angiogenesis (Gschwind *et al*, 2004). The epidermal growth factor receptor has thus been identified as an important target in cancer therapy (Baselga and Arteaga, 2005). Several agents, including small-molecule inhibitors of the tyrosine kinase activity of EGFR (EGFR-TKIs) and monoclonal antibodies (mAbs) specific for EGFR, have been designed to block EGFR signalling selectively (Ettinger, 2006; Harari and Huang, 2006; Imai and Takaoka, 2006). Among EGFR-TKIs, gefitinib and erlotinib have been extensively evaluated in non-small cell lung cancer (NSCLC), and sensitivity to these drugs has been associated with the presence of somatic mutations in the EGFR kinase domain or with *EGFR* amplification (Lynch *et al*, 2004; Paez *et al*, 2004; Pao *et al*, 2004; Cappuzzo *et al*, 2005; Mitsudomi *et al*, 2005; Takano *et al*, 2005). Various mAbs to EGFR are also undergoing preclinical and clinical trials of their efficacy as anticancer agents. However, biological markers able to predict the response to such antibodies have remained elusive.

The possibility of combining chemotherapy or radiation therapy with anti-EGFR mAb treatment has generated much interest, because the cellular targets for these agents and their mechanisms of action are different (Baumann and Krause, 2004). Studies have thus been undertaken to determine whether inhibition of EGFR signalling improves the response to chemotherapy or radiation therapy. Preclinical studies have shown that the anti-EGFR mAb cetuximab markedly increases the cytotoxic effect of chemotherapy or radiation therapy in various EGFR-expressing tumour cell lines (Huang *et al*, 1999; Milas *et al*, 2000; Buchsbaum *et al*, 2002; Prewett *et al*, 2002; Raben *et al*, 2005; Ettinger, 2006). A phase III clinical trial also showed that the combination of cetuximab with

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