are highly contaminated with normal cells. Secondly, *EGFR* mutation analysis based on DNA sequencing requires special instruments and is also time-consuming and expensive. Therefore, some simple and highly sensitive nonsequencing methods to detect *EGFR* mutations have been reported (10–22). However, the accuracy of these methods for clinical use have not been assessed in prospective studies.

High-resolution melting analysis (HRMA) using the LCGreen I (Idaho Technology) dye was introduced as an easy, quick, and inexpensive method for the screening of mutations (23), and we established and validated the HRMA method to detect DEL and L858R mutations in cases of NSCLC (9, 10). Our cell line study revealed that DEL and L858R mutations could be detected using HRMA in the presence of 10% and 0.1% of mutant cells, respectively (10). We also showed that the two major mutations could be identified by HRMA retrospectively using DNA extracted from archived Papanicolaou-stained cytologic slides with 88% sensitivity and 100% specificity (9). Furthermore, it was shown that among patients treated with gefitinib, the response rate (78% versus 8%), time-to-progression (median, 9.2 versus 1.6 months), and overall survival (median, 21.7 versus 8.7 months) were significantly better in patients with *EGFR* mutations than with wild-type *EGFR* ($P < 0.001$), as detected by HRMA (9). These results suggest that this easy, quick, and inexpensive method which was done using diagnostic small samples of advanced NSCLC tumors is one of the most useful and precise methods to detect *EGFR* mutations in clinical practice.

In this study, we designed a prospective study to detect two major *EGFR* mutations by HRMA using small diagnostic cytologic or biopsy specimens and surgically resected specimens, and the results were compared with the results of DNA sequencing methods combined with LCM, which we consider as the "gold standard" for such detection, applied to methanol-fixed, paraffin-embedded surgically resected specimens. We evaluated the diagnostic sensitivity, specificity, predictive values, and accuracy of the detection of *EGFR* mutations using HRMA and revealed that this method is feasible for clinical use to detect *EGFR* mutations in small samples obtained from patients with NSCLC.

Patients and Methods

Patients and materials. Patients with lung lesions, which were suspected clinically to be operable NSCLC, were enrolled in this prospective study. The patients were scheduled for bronchoscopy or percutaneous needle biopsy to establish the histologic diagnosis, and informed consent was obtained from each of the patients prior to these diagnostic procedures. Thereafter, the patients diagnosed with NSCLC underwent lung surgery at our hospital. In this study, mutational analysis of *EGFR* was done by HRMA or DNA sequencing methods combined with LCM in all the patients in which both the preoperatively obtained diagnostic specimens and the resected specimens were histologically confirmed by a certified pathologist to contain malignant cells.

Based on a protocol approved by the Institutional Review Board of the National Cancer Center, we did mutational analyses of *EGFR* to detect DEL and L858R in the eligible patients. The Papanicolaou-stained cytologic slides ($n = 35$), formalin-fixed, paraffin-embedded transbronchial or percutaneous needle biopsy specimens ($n = 34$), and methanol-fixed, paraffin-embedded surgically resected specimens subjected to LCM using a PixCell II LCM system (Arcturus Engineering, Inc.; $n = 52$) were collected prospectively. DNA was extracted using the QIAamp DNA Micro Kit (Qiagen), as described in our previous report (10).

HRMA. PCR was done to amplify exons 19 or 21 of *EGFR* using LCGreen I (Idaho Technology) on a LightCycler (Roche Diagnostics) and primers designed as previously described (10). If the first PCR products were not available for the mutational analyses of the melting curves, we did a second PCR using the same primers. These PCR products were denatured at 95°C for 10 min and cooled to 40°C to promote the formation of heteroduplexes. The LightCycler capillary was transferred to an HR-1 (Idaho Technology), an HRMA instrument, and heated at a transition rate of 0.3°C/s. Data were acquired and analyzed using the accompanying software (Idaho Technology). After normalization and temperature-adjustment steps, melting curve shapes from 78.5°C to 85.5°C were compared between the tumor samples and control samples. Human Genomic DNA (Roche Diagnostics) was used as the negative control sample with wild-type *EGFR*. Samples revealing skewed or left-shifted curves as compared with the control samples were judged to have mutations without positive controls (9, 10). All analyses were done in a blinded fashion by two researchers (T. Fukui and T. Takano). After independent evaluation by the two researchers, the final judgment was arrived at by consensus after joint viewing of the melting curves from both.

DNA sequencing methods with LCM. In our previous study, we did a direct sequencing or pyrosequencing of *EGFR* in patients with recurrent NSCLC after primary surgery (5). Based on the results of our previous study, we consider direct sequencing with LCM for the detection of DEL and pyrosequencing with LCM for the detection of L858R as the gold standard in relation to *EGFR* mutational analysis. DNA was extracted from methanol-fixed, paraffin-embedded surgical specimens by LCM, according to a previously described method (24). Direct sequencing of the PCR products for DEL was done using ABI PRISM3700 and 3100 DNA sequencers (Applied Biosystems). Pyrosequencing to analyze L858R was done using Pyrosequencing PSQ 96MA (Pyrosequencing; refs. 5, 25). The *EGFR* mutational analysis using DNA sequencing methods was done in a blinded fashion by a researcher (H. Sakamoto) according to a previously described method (5), and then compared with the corresponding results obtained using HRMA.

Statistical analysis. The primary end point of this study was the sensitivity and specificity of the results obtained using HRMA as compared with those of the results obtained using DNA sequencing with LCM. The sample size was calculated using a statistical power level of 0.80 and two-sided α level of 0.1 on the basis of an estimated sensitivity of at least 0.80 and an expected value of 0.95 for HRMA, a minimum of 20 patients with *EGFR*-mutated tumors were required. Because the percentage of NSCLC patients with *EGFR* mutations was expected to be 40% in this study population composed of only Japanese, approximately 50 patients with NSCLC were needed. Therefore, considering a specificity of at least 0.80 and the expected value of 0.95 for HRMA, 30 patients with wild-type tumors showed a statistical power level of 0.90 using a two-sided α level of 0.1.

The associations between mutational status and patient characteristics were assessed by a χ^2 test using the SPSS statistical package (SPSS version 11.0 for Windows; SPCC, Inc.).

Results

Patient characteristics. From December 2005 to December 2006, 92 patients with clinically suspected operable NSCLC were enrolled in this study. The following diagnostic procedures were done preoperatively in 90 patients: bronchoscopy ($n = 57$), percutaneous needle biopsy ($n = 27$), or bronchoscopy followed by percutaneous needle biopsy ($n = 6$). The patient characteristics are shown in Table 1. All the patients were Japanese. Among the patients, a definitive diagnosis was established in 85 patients by bronchoscopy in 43 of 59 patients

Table 1. Patient characteristics

(A) Characteristics of all the patients enrolled in this study (n = 92)

	All (n = 92)	BF (n = 64)	PNB (n = 34)*
Age, year, median (range)	64 (34-84)	64 (38-84)	62 (41-79)
Gender (male/female)	58/34	41/23	23/11
Smoking history (N/F/C)	29/30/33	23/19/22	7/14/13
Tumor size, mm, average (range)	27.2 (10.2-73.4)	28.3 (13.8-56.6)	24.5 (10.2-73.4)
Accuracy of the diagnostic procedure (%)	66/85 (77.6)	43/59 (72.9)	25/31 (80.6)
Accuracy of the cytologic slides (%)	54/85 (63.5)	31/59 (52.5)	23/30 (76.7)
Accuracy of the biopsy specimens (%)	42/62 (67.7)	35/54 (64.8)	7/9 (77.8)

(B) Characteristics of the patients who underwent analysis of the EGFR mutations in this study (n = 52)

	All (n = 52)	BF (n = 38)	PNB (n = 17)†
Age, year, median (range)	64.5 (34-84)	64.5 (34-84)	64 (47-78)
Gender (male/female)	36/16	25/13	14/3
Smoking history (N/F/C)	16/17/19	15/11/12	1/7/9
Tumor size, mm, average (range)	27.0 (11.0-56.6)	28.3 (20.6-56.6)	24.1 (11.0-48.8)
Postoperative diagnosis (Ad/Sq/LCNEC)	45/5/2	34/4/0	12/3/2
Pathologic stage (IA/B, IIA/B, IIIA/B)	19/13, 3/5, 9/2	15/8, 3/2, 8/2	7/5, 0/2, 3/0

NOTE: Never smokers were defined as patients who had never smoked, former smokers were defined as patients who had stopped smoking at least 1 y before the diagnosis, and current smokers were defined as patients who were still smoking at the time of the diagnosis.
Abbreviations: BF, bronchoscopy; PNB, percutaneous needle biopsy; N, never smoker; F, former smoker; C, current smoker; Ad, adenocarcinoma; Sq, squamous cell carcinoma; LCNEC, large cell neuroendocrine carcinoma.
*Including six patients in whom bronchoscopy was done followed by percutaneous needle biopsy.
†Including three in whom bronchoscopy was done followed by percutaneous needle biopsy.

(72.9%) and by percutaneous needle biopsy in 25 of 31 patients (80.6%); in 18 of the 85 (21.2%) patients, the histologic diagnosis could not be established preoperatively by bronchoscopy and/or percutaneous needle biopsy, the patients underwent lung surgery for suspicious malignant lung lesion, and examination of the resected specimens revealed the diagnosis of primary NSCLC in 17 and malignant lymphoma in 1 of the 18 patients. Among the 76 patients diagnosed to have primary NSCLC, 73 consented to undergo lung surgery. Finally, the analysis for *EGFR* mutations was done on 52 patients with a definitive histologic diagnosis of primary NSCLC, established both by examination of the preoperative diagnostic specimens and of the corresponding resected specimens (Fig. 1).

Mutational analyses. We analyzed 35 cytologic samples and 34 biopsy specimens obtained from 52 patients by HRMA, and

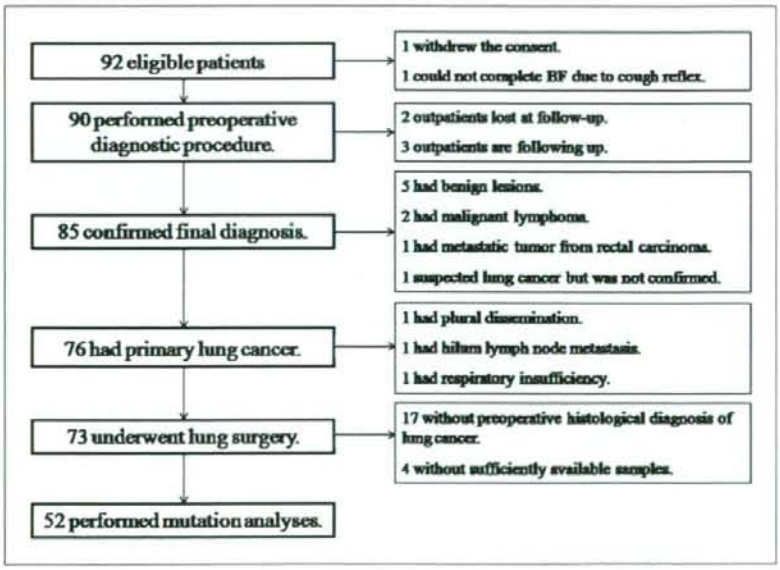

Fig. 1. Flowchart of the analyses conducted in 92 enrolled patients with lung tumors in this study.

Table 2. EGFR mutation status among the patient subgroups

	n	*EGFR* mutations*				*P*
		DEL	L858R	Total	%	
Total	52	5	13	18	34.6	—
Gender						
Women	16	2	9	11	68.8	0.001
Men	36	3	4	7	19.4	
Smoking history						
Never	16	3	8	11	68.8	0.001†
Former	17	2	4	6	35.3	
Current	19	0	1	1	5.3	
Histology						
Ad	44	5	13	18	100	0.025‡
Sq	6	0	0	0	0	
LCNEC	2	0	0	0	0	

Abbreviations: DEL, deletional mutations in exon 19; L858R, a point mutation at codon 858 in exon 21; Ad, adenocarcinoma; Sq, squamous cell carcinoma; LCNEC, large cell neuroendocrine carcinoma.
*The EGFR mutations were analyzed by DNA sequencing with LCM.
†Comparison between never smokers and others.
‡Comparison between adenocarcinoma and others.

did both HRMA and DNA sequencing with LCM in the 52 resected specimens corresponding to the 52 patients. Among the 52 surgically resected specimens analyzed by DNA sequencing with LCM, there were 18 (34.6%) samples with *EGFR* mutations, 5 with DEL mutations, and 13 with L858R mutations. As shown in Table 2, the *EGFR* mutations were detected more frequently in women, never-smokers, and patients with a histologic diagnosis of adenocarcinoma. All results from HRMA done in a blinded fashion by two researchers (T. Fukui and T. Takano) were consistent.

HRMA could be conducted using small diagnostic samples from all 52 patients, although the analysis needed to be conducted using the second PCR product in 15 cases. In the analysis of exon 19, 5 samples revealed different curves from the control and 47 samples revealed almost the same curves as the control; therefore, we judged that the five former patients had DEL mutations (Fig. 2A). In the analysis of exon 21, 10 samples revealed a left-shift from the control and 42 samples revealed almost the same curves as the control; therefore, we judged that the 10 former patients had L858R mutations

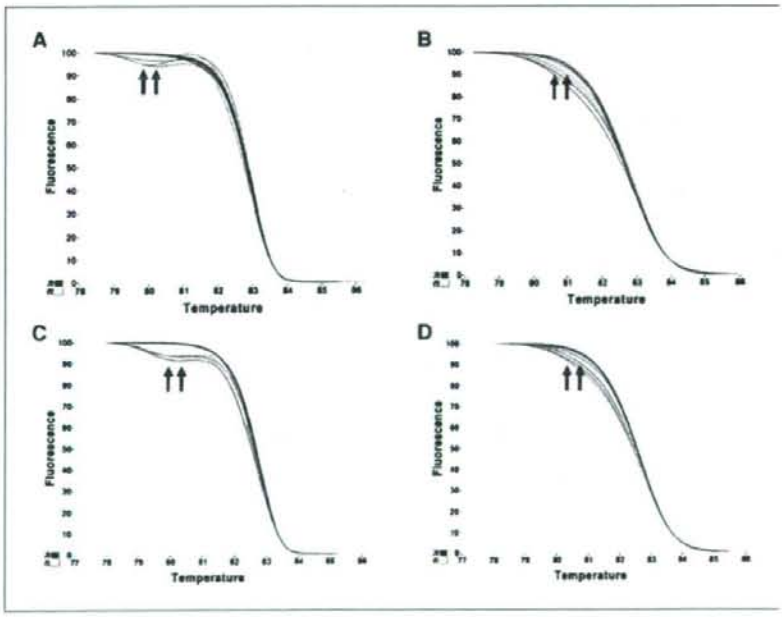

Fig. 2. Adjusted melting curves obtained by HRMA of the samples in this study to detect *EGFR* mutations (↑), in-frame deletions in exon 19 (*A*, small samples; *C*, resected specimens) and a point mutation in exon 21 (*B*, small samples; *D*, resected specimens). Each sample that revealed a skewed or left-shifted curve from those of the control sample was judged to have a mutation.

Table 3. Results of the EGFR mutation analyses in patients with *EGFR* mutation-positive tumors

No. of patients	Small samples	Surgically resected specimens	
	HRMA	HRMA	Sequence with LCM
13	DEL	DEL	DEL1*
26	DEL	DEL	DEL1*
32	DEL	DEL	DEL2†
40	DEL	DEL	DEL2†
47	DEL	DEL	DEL1*
5	L858R‡	L858R	L858R
6	Wild-type	L858R	L858R
12	L858R	L858R	L858R
18	L858R	L858R	L858R
21	L858R	L858R	L858R
23	L858R‡	L858R	L858R
25	Wild-type	L858R	L858R
27	L858R‡	L858R	L858R
28	L858R	L858R	L858R
31	Wild-type‡	L858R	L858R
41	L858R‡	L858R	L858R
53	L858R	L858R	L858R
54	L858R‡	L858R	L858R

Abbreviations: DEL, deletional mutations in exon 19; L858R, a point mutation at codon 858 in exon 21.
*DEL1: del E746-A750 (del 2235-2249).
†DEL2: del E746-A750 (del 2236-2250).
‡The analyses by HRMA were done using second PCR products.

(Fig. 2B). All the 52 surgically resected specimens analyzed by DNA sequencing with LCM could also be analyzed by HRMA, although the analysis needed to be conducted using the second PCR product in two cases. DEL mutations were detected in 5 patients (Fig. 2C) and L858R mutations in 13 patients (Fig. 2D) among the 52 patients. Of the 52 specimens, both cytologic slides and biopsy specimens were analyzed in 17 cases. Discrepant results were obtained by HRMA in one of the cases, with L858R mutation being detected in the cytologic slides but not in the biopsy specimens. We included this patient in the population with L858R mutations.

The results of HRMA were consistent with the results of DNA sequencing with LCM in all the surgically resected specimens analyzed by the two methods. On the other hand, HRMA using small diagnostic specimens revealed the wild-type curve in three cases, although analysis of the corresponding surgically resected specimens analyzed by pyrosequencing with LCM revealed the L858R mutation (Table 3). Thus, the results for these samples obtained by HRMA were considered as false-negative results. Neither method of analysis yielded any false-positive cases. The results of the *EGFR* mutational analysis by HRMA compared with DNA sequencing with LCM using surgically resected specimens were shown in Table 4. The sensitivity, specificity, and accuracy of HRMA using small diagnostic specimens were 83.3%, 100%, and 94.2%, respectively. Using surgically resected specimens, those of HRMA were all 100%.

Discussion

In this prospective study, we showed the high accuracy of the HRMA method for detecting two major *EGFR* mutations, DEL and L858R in patients with NSCLC. The accuracy of HRMA was clearly equal to that of DNA sequencing with LCM for the detection of mutations in surgically resected specimens. On the other hand, the sensitivity and specificity of HRMA were 83.3% (90% confidence interval: 68.9-97.7%) and 100%, respectively, when the small diagnostic samples were analyzed. Although the sensitivity of HRMA which was estimated to be at least 0.80 did not reach statistical significance, we consider HRMA as one of the available methods for the detection of *EGFR* mutations in clinical practice because the specificity, which is important for clinical decision-making, of HRMA was 100% and the *EGFR* mutation rate was less than the expected 40% to secure enough statistical power in this study.

Recently, many researchers reported establishing simple and highly sensitive nonsequencing methods for detecting *EGFR* mutations using small tumor samples (11-22), and the results of several mutation analyses were correlated with the clinical outcome of EGFR tyrosine kinase inhibitor treatment (17-19). Using serial dilution studies, some researchers have reported methods that are able to detect mutations in samples containing ~0.1% to 10% mutated DNA (13, 14, 16-18, 20-22), as opposed to direct DNA sequencing which requires the presence of at least 10% to 30% of mutated DNA in the samples (18, 20). Additionally, several novel methods offered higher sensitivity and specificity than DNA sequencing to identify the mutations in clinical samples. But almost none of the methods were validated for diagnostic accuracy in a prospective study, and we therefore consider these methods to still be unsuitable for routine clinical examination. Although these nonsequencing methods were not mutually compared, based on our previous results of retrospectively verifying the accuracy of HRMA (9, 10), we thought to develop in this prospective study an easy, quick (PCR for ~1 hour and HRMA for 2 to 3 minutes), and inexpensive (at a running cost per sample of approximately $7.50, which consisted of $5.50 for the DNA extract and less than $2.00 for PCR using LCGreen I dye) method that might be useful in clinical practice with a great advantage over DNA sequencing, which requires the

Table 4. Comparison of the sensitivity, specificity, predictive values, and accuracy between HRMA and DNA sequencing with LCM (n = 52)

	HRMA using small samples	HRMA using surgically resected specimens
True-positive	15	18
True-negative	34	34
False-positive	0	0
False-negative	3	0
Sensitivity	83.3 (68.9-97.8)	100
Specificity	100	100
NPV	91.9 (84.5-99.3)	100
PPV	100	100
Accuracy	94.2 (88.9-99.5)	100

NOTE: The results of these analyses were compared with those of DNA sequencing with LCM (used as the gold standard in this study). Data are presented as % or % (90% confidence interval). True-positive is defined as the correct detection of DEL in exon 19 or L858R in exon 21.
Abbreviations: NPV, negative predictive value; PPV, positive predictive value.

Table 5. Results of HRMA using cytologic slides or biopsy specimens

	Cytologic slides (n = 35)		Biopsy specimens (n = 34)	
	First PCR	Second PCR	First PCR	Second PCR
Successfully analyzed	29 (83.0%)	35 (100%)	5 (15.0%)	34 (100%)
True-positive	7	11	1	10
True-negative	19	21	4	22
True-negative	0	0	0	0
False-positive	3	3	0	2
Sensitivity	70.0% (7/10)	78.6% (11/14)	100% (1/1)	83.3% (10/12)
Specificity	100% (19/19)	100% (21/21)	100% (4/4)	100% (22/22)
NPV	100% (7/7)	100% (11/11)	100% (1/1)	100% (10/10)
PPV	86.4% (19/22)	87.5% (21/24)	100% (4/4)	91.2% (22/24)
Accuracy	89.7% (26/29)	91.4% (32/35)	100% (5/5)	94.1% (32/34)

NOTE: The results of these analyses were compared with those of DNA sequencing with LCM (used as the gold standard in this study). True-positive is defined as the correct detection of DEL in exon 19 or L858R in exon 21.
Abbreviations: NPV, negative predictive value; PPV, positive predictive value.

extraction of high-quality DNA from an adequate amount of pure tumor cells, takes a long time, and is expensive.

In this study, the three patients with L858R detected by DNA pyrosequencing with LCM using the surgically resected specimens were labeled as having the wild-type *EGFR* in the analyses conducted using the small diagnostic samples. With regard to these false-negative results, the following three points need to be discussed: first, our previous study, conducted using human lung cancer cell lines, showed that HRMA can detect the mutations, even when samples contain only a small proportion (DEL, 10%; L858R, 0.1%) of mutant cells (10). In this study, the sensitivity of HRMA was also considered to be sufficiently high for the detection of *EGFR* mutations, especially L858R, even when the analysis was conducted using small samples after evaluation by a clinical pathologist to determine if they contained benign or malignant cells. Thus, we assume a higher accuracy of HRMA when using small samples in clinical practice. Although it still needs to be comparatively analyzed with the previously reported non-sequencing methods, HRMA can be considered as one of the sensitive methods available for the detection to *EGFR* mutations in clinical practice.

Second, high-quality DNA should be preserved in clinical samples to obtain the best results. There always remains the risk of an indeterminate or false-negative result because the DNA might have degenerated during sampling or during the preservation of clinical samples. In a comparison between the cytologic slides and biopsy specimens, better results were obtained from analyses of the first PCR products using the cytologic slides rather than the results obtained using the biopsy specimens, regardless of the amount of tumor cells examined (Table 5). This could probably be explained by the differences in the method of sample fixation between the two types of specimens. It has been suggested by a previous report that DNA is preserved better in the methanol-fixed samples than in the formalin-fixed specimens (26). Therefore, if we used methanol for specimen fixation of biopsy specimens, the results of HRMA using the first PCR products from small biopsy samples might improve. Hereafter, we propose to perform mutation analyses using methanol-fixed specimens, if possible.

Finally, we need to consider the possibility of intratumoral heterogeneity, and small diagnostic samples and surgically resected specimens may each represent overlapping but different populations of these tumor cells. A lack of association in the immunohistochemical expression profile between lung biopsy specimens and the corresponding resected tumor specimens has been reported (27). Furthermore, intratumoral heterogeneity was shown not only in terms of microheterogeneity of the tumor cell phenotype (28), but in terms of genetic heterogeneity in cancer (29, 30). In particular, the intratumoral genetic heterogeneity of *EGFR* mutations may explain the variable clinical response of NSCLC to gefitinib. It is also possible that the small diagnostic samples contain only wild-type cells, even if the tumor, overall, shows mutations, because the small samples yield only small part of the tumor. It is always necessary to consider the possibility of a false-negative result of mutational analyses conducted using the small samples.

In the current prospective study, we showed the feasibility and high accuracy of using HRMA for detecting two major *EGFR* mutations, DEL and L858R, in patients with NSCLC. Although HRMA showed high accuracy, the possibility of indeterminate or false-negative results, and because of the sensitivity of this method, the quality of DNA preservation in the clinical samples or intratumoral genetic heterogeneity, must be borne in mind to a certain extent when this analysis is conducted using small diagnostic samples. Therefore, HRMA should not be used to exclude patients from EGFR tyrosine kinase inhibitor treatment on the basis of the negative results only. Based on the results of this prospective study, we suggest that this method is very useful for clinical decision-making, especially in patients with a positive result.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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thus influenced the results including those assessed (overall survival) and not assessed (disease free survival and time to progression). Future studies on the efficacy of docetaxel as a second line agent should serve to address issues like the optimal dose regimen and intensity as well as adjust for potential confounders.

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Reply: Higher Intensity Does Not Necessary Yield Better Survival in Second-Line Chemotherapy for NSCLC

To the Editor:

We would like to thank Singh et al. for suggesting that the dose of docetaxel and previous treatment modality may have an impact on second-line therapy in non-small cell lung cancer (NSCLC). Herein, we discuss the dose of docetaxel and the influence of previous chemotherapy in relation to second-line treatment of NSCLC.

In second-line chemotherapy for NSCLC, whether a higher dose of an anticancer agent would inevitably yield a longer survival is open to question. In a study comparing docetaxel 100 mg/m^2, docetaxel 75 mg/m^2 and best supportive care, the overall survivals were 5.9, 7.5, and 7.0 months, respectively.[1] Docetaxel 100 mg/m^2 was also found to be inferior to docetaxel 75 mg/m^2 in terms of the 1-year survival rate in another phase III study.[2] A similar tendency was also observed for another agent in the second-line setting; pemetrexed 500 mg/m^2 and 900 mg/m^2 were compared, and the overall median survivals were 6.7 and 6.9 months, respectively, and the hazard ratio was 1.013 (95% confidence interval, 0.837–1.226).[3] Even the response rate in the 900 mg/m^2 arm did not exceed that in the 500 mg/m^2. Thus, finding the optimal dose of docetaxel or other agents for second-line chemotherapy may be an intriguing issue.[4]

Meanwhile, docetaxel 60 mg/m^2 is the standard therapeutic dose in Japan, since a Japanese phase I trial determined the maximum tolerated dose to be 70 mg/m^2.[5] Even though this dose of docetaxel is lesser than that used in other countries, this may be the optimal dose for Japanese. In a phase II study of docetaxel for previously untreated NSCLC conducted in Japan, the response rate to docetaxel 60 mg/m^2 was 19%, no less than that to the higher doses used in other countries.[6] A retrospective study evaluating docetaxel 60 mg/m^2 for previously treated NSCLC also showed a response rate of 18.5%, comparable with that reported for higher doses.[7] This difference in the dose requirement in Japanese may be attributed to ethnic differences between the Japanese and other populations, but the issue remains under debate.

The previously employed treatment modality differed between those who had received a combination of carboplatin and paclitaxel (group P) and those who had received a combination of a platinum and an agent other than paclitaxel [group nonpaclitaxel (NP)] in our study. We consider, however, that this difference had only a small impact on our study results, for three reasons. Firstly, all the patients in our study had metastatic disease at the time of recurrence and start of docetaxel therapy. Secondly, although 29% of patients in group NP had received radiotherapy, the response rate to the previous treatment in group NP was the same as that in group P (45.0 versus 44.9%, respectively). In general, the response rate to chemoradiotherapy is higher than that to chemotherapy alone. This difference may have disappeared in our study, probably because we only recruited patients who developed recurrence after chemoradiotherapy. Finally, no previous studies of second-line chemotherapy for NSCLC have dealt with these issues. Even though multiple modalities may have been used in previous treatment, we can only evaluate the integrated result of the treatment. It is impossible to distinguish between the efficacy of chemotherapy and radiotherapy if both are undertaken simultaneously.

In conclusion, further investigation of the optimal dose of chemotherapeutic agents for second-line chemotherapy of NSCLC is warranted. The efficacy of previous chemotherapy, whether or not administered in combination with radiotherapy, is a useful reference for subsequent docetaxel therapy.

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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

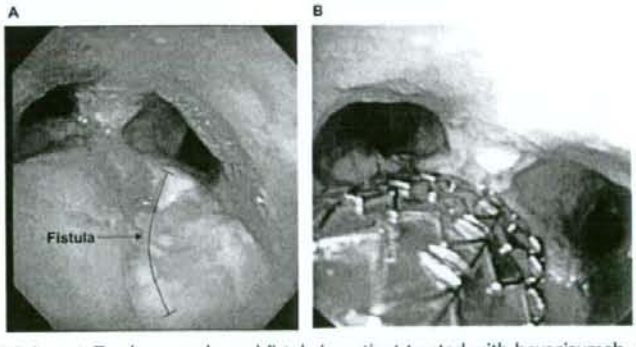

FIGURE 1. *A*, Tracheo-esophageal fistula in patient treated with bevacizumab. *B*, Coated stent in the esophagus, as visualized through the large posterior airway defect.

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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.

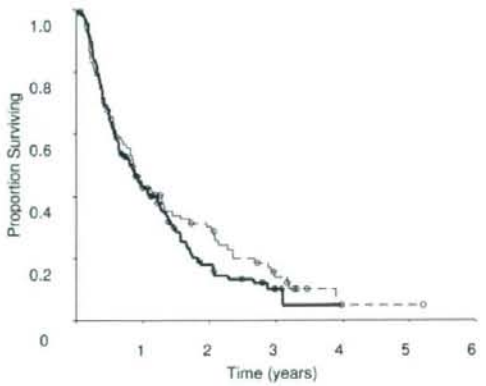

FIGURE 1. Overall survival classified by the previous chemotherapy regimens. Continuous line: carboplatin and paclitaxel (group P, n = 123); and dotted line: platinum and an agent other than paclitaxel (group NP, n = 97). Hazard ratio (95% confidence interval): 1.09 (0.81–1.47).

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

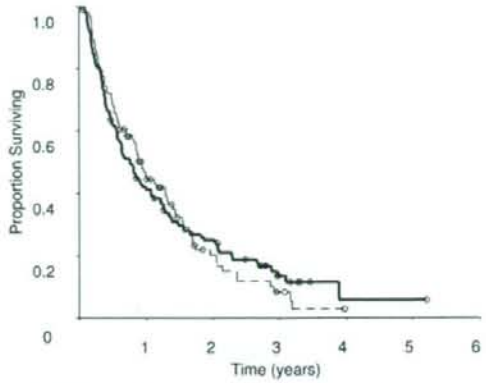

FIGURE 2. Overall survival classified by the responses to previous chemotherapy. Continuous line: SD/PD (n = 118); and dotted line: CR/PR (n = 102). Hazard ratio (95% confidence interval): 0.91 (0.68–1.23).

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).

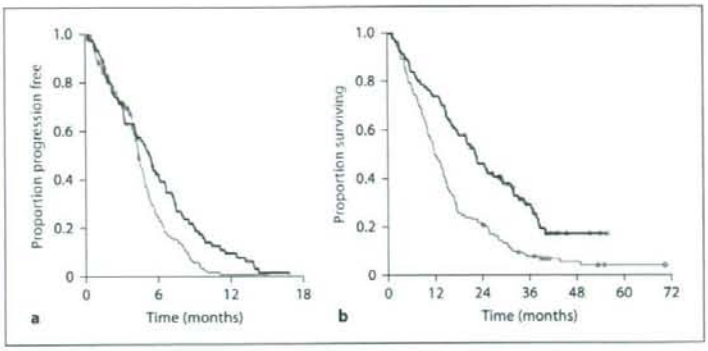

Fig. 1. PFS (**a**) and overall survival (**b**) in all patients. Thick line = Female patients; thin line = male patients.

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

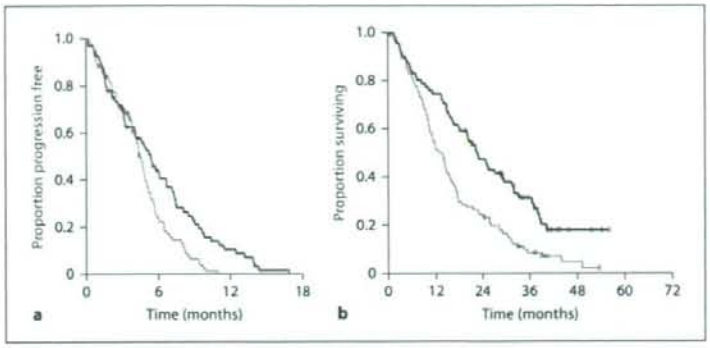

Fig. 2. PFS (**a**) and overall survival (**b**) in patients with adenocarcinoma. Thick line = Female patients; thin line = male patients.

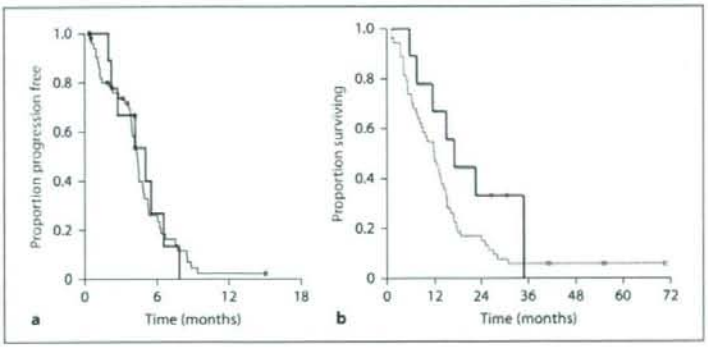

Fig. 3. PFS (**a**) and overall survival (**b**) in patients with non-adenocarcinoma. Thick line = Female patients; thin line = male patients.

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