Table 1. Patient Characteristics

Characteristic	BSA-Based Arm No. of Patients	%	Individualized Arm No. of Patients	%	P
Enrolled	30		29		
Eligible	30	100	29	100	
Age, years					.62
Median	61		62		
Range	52-73		45-73		
Sex					
Male	25	83	19	66	.14
Female	5	17	10	34	
ECOG PS					
0	7	23	1	3	.08
1	22	73	26	90	
2	1	3	2	7	
Prior treatment					
None	4	13	4	14	.99
Surgery	11	37	9	31	.65
Radiotherapy	13	43	10	34	.49
Chemotherapy	21	70	18	62	.52
Platinum-based regimens	20	67	16	55	.37
Site of disease					
Lung	23	77	28	97	.10
Liver	0	0	2	7	.24
Pleura	8	27	12	41	.23
Bone	7	23	9	31	.71
Extrathoracic lymph nodes	0	33	10	34	.93
Laboratory parameters					
ALB, g/L					.02
Median	38		35		
Range	26-45		24-44		
AAG, g/L					.04
Median	1.00		1.25		
Range	0.28-2.15		0.64-2.54		
AST, U/L					.67
Median	21		22		
Range	10-40		7-41		
ALT, U/L					.88
Median	18		18		
Range	6-54		4-45		
ALP, U/L					.03
Median	249		324		
Range	129-540		185-986		

Abbreviations: ECOG, Eastern Cooperative Oncology Group; PS, performance status, ALB, serum albumin; AAG, alpha-1-acid glycoprotein; ALP, serum alkaline phosphatase.

AUC was significantly smaller in the individualized arm than in the BSA-based arm ($P < .01$; Fig 3).

PD

In both arms, neutropenia was the predominant toxicity related to docetaxel treatment, and 28 of 30 (93%) patients in the BSA-based arm and 25 of 29 (86%) patients in the individualized arm had grade 3 or 4 neutropenia.

Table 2. Docetaxel PK Parameters

Parameters	BSA-Based Arm (n = 30)	Individualized Arm (n = 29)
C_{max}, μg/mL	0.36-2.70	0.99-2.41
$t_{1/2}$ alpha*, minutes	9.2 ± 3.3	9.2 ± 2.7
$t_{1/2}$ beta*, hours	5.0 ± 4.8	7.4 ± 11.7
CL* L/h	37.6 ± 6.3	34.8 ± 7.1
CL* L/h/m²	22.6 ± 3.4	22.1 ± 3.4
AUC		
Mean mg/L · h	2.71	2.64
Range mg/L · h	2.02-3.40	2.15-3.07
Median	2.65	2.66
SD	0.40	0.22

Abbreviations: PK, pharmacokinetic; BSA, body-surface area; CL, clearance; AUC, area under concentration-time curve; SD, standard deviation. *Data represent mean ± SD.

Nonhematologic toxicities, such as gastrointestinal and hepatic toxicities (ie, hyperbilirubinemia, aminotransferase elevations), were mild in both arms.

PD effects shown as the percentage decrease in ANC are listed in Table 3. The percentage decrease in ANC for the BSA-based arm and individualized arm were 87.1% (range, 59.0 to 97.7%; SD, 8.7) and 87.5% (range, 78.0 to 97.2%; SD, 6.1), respectively, suggesting that the interpatient variability in the percentage decrease in ANC was slightly smaller in the individualized arm than in the BSA-based arm (Fig 4). The response rates between the two arms were similar; five of 30 (16.7%) and four of 29 (13.8%) patients

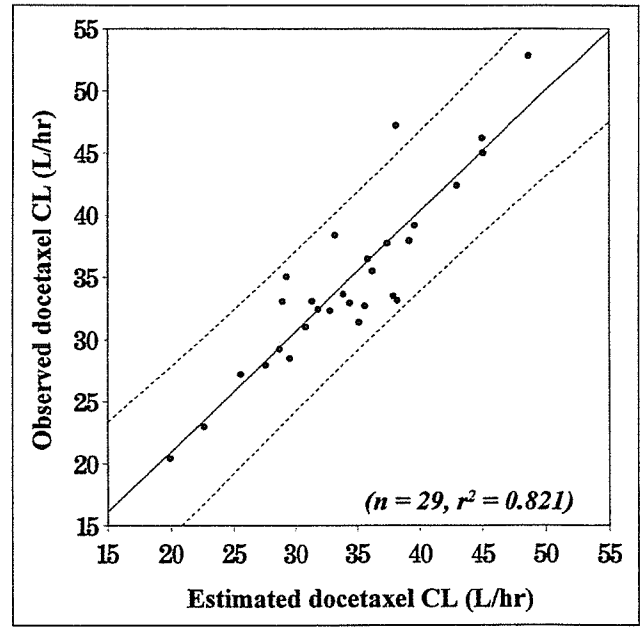

Fig 2. Correlation between the estimated and observed docetaxel clearance (CL) in the individualized arm (n = 29). (—) Linear regression line (r^2 = 0.821); (– – – –) 95% CIs for individual estimates.

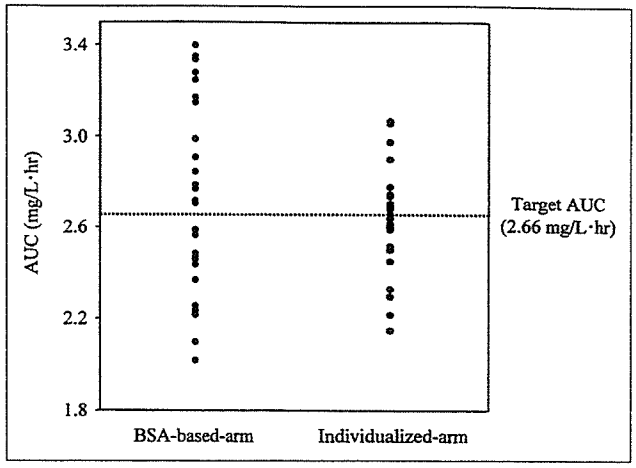

Fig 3. Comparison of area under the concentration-time curve (AUC) variability between the arms ($P < .01$; F test). BSA, body-surface area.

achieved a partial response in the BSA-based arm and individualized arm, respectively.

DISCUSSION

In oncology practice, the prescribed dose of most anticancer drugs is currently calculated from BSA of individual patients to reduce the interpatient variability of drug exposure. However, PK parameters, such as CL of many anticancer drugs, are not related to BSA.[2,39-43] Although PK parameters of docetaxel are correlated with BSA, individualized dosing based on individual metabolic capacities could further decrease the interpatient variability.[43]

CYP3A4 plays an important role in the metabolism of many drugs, including anticancer agents such as docetaxel, paclitaxel, vinorelbine, and gefitinib. This enzyme exhibits a large interpatient variability in metabolic activity, accounting for the large interpatient PK and PD variability. We have developed a novel method of estimating the interpatient variability of CYP3A4 activity by urinary metabolite of exogenous cortisol. That is, the total amount of 24-hour urinary 6-β-OHF after cortisol administration was highly correlated with docetaxel CL. We conducted a prospective randomized PK and PD study of docetaxel to evaluate whether the application of our method to individualized dosing could decrease PK and PD variability compared with BSA-based dosing.

Table 3. Percentage Decrease in ANC

Parameters	BSA-Based Arm (n = 30)	Individualized Arm (n = 29)
Percentage decrease in ANC, %		
Mean	87.1	87.4
Range	59.0-97.7	78.0-97.2
Median	89.7	88.4
SD	8.7	8.1

Abbreviations: ANC, absolute neutrophil count; BSA, body-surface area; SD, standard deviation.

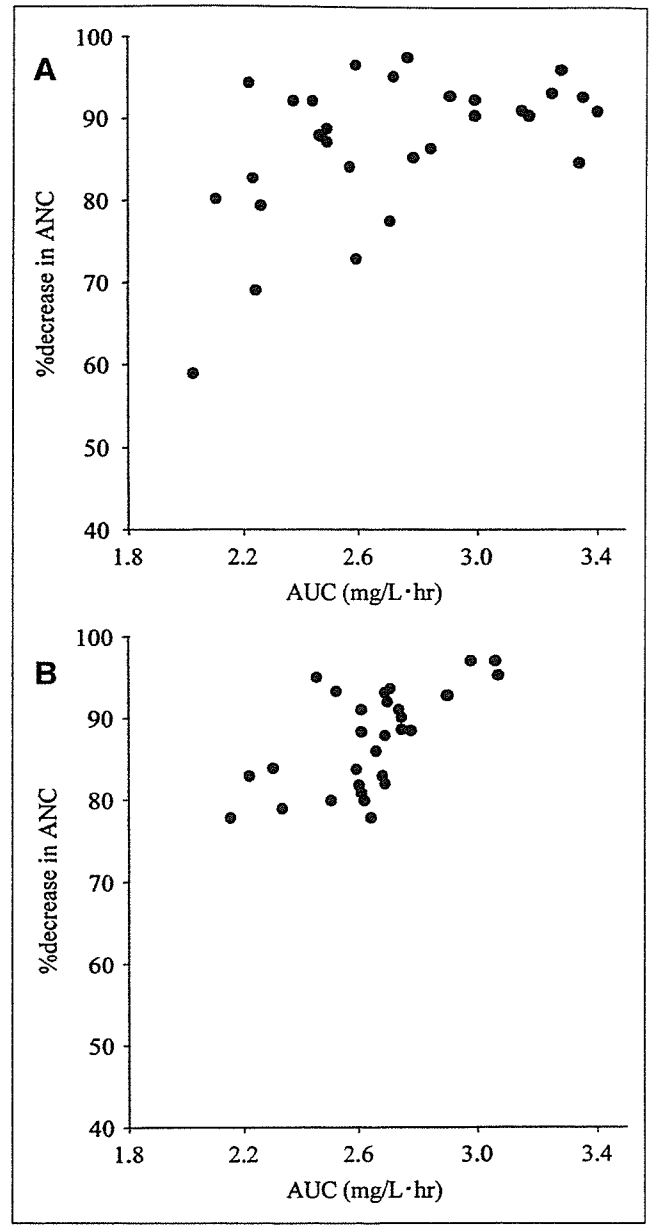

Fig 4. Correlation between area under the concentration-time curve (AUC) and percentage decrease in absolute neutrophil count (ANC) in each arm. (A) body-surface area–based arm; (B) individualized arm.

The study by Hirth et al[28] showed a good correlation between the result of the erythromycin breath test and docetaxel CL, and the study by Goh et al[29] showed a good correlation between the midazolam CL and docetaxel CL. In our study, we prospectively validated the correlation between docetaxel CL and our previously published method using the total amount of urinary 6-β-OHF after

cortisol administration in the individualized arm. As shown in Fig 2, the observed docetaxel CL was well estimated, and the equation for the estimation of docetaxel CL developed in our previous study was found to be reliable and reproducible. The target AUC in the individualized arm was set at 2.66 mg/L · h. This value was the mean value from our previous study, in which 29 patients were treated with 60 mg/m^2 of docetaxel. Individualized doses of docetaxel ranged from 37.4 to 76.4 mg/m^2 and were lower than expected.

The SD of AUC in the individualized arm was about 46.2% smaller than that in the BSA-based arm, a significant difference; this result seems to indicate that the application of our method to individualized dosing can reduce the interpatient PK variability. Assuming that the variability of AUC could be decreased 46.2% by individualized dosing applying our method, overtreatment could be avoided in 14.5% of BSA-dosed patients by using individualized dosing (Fig 5, area A), and undertreatment could be avoided in another 14.5% of these patients (Fig 5, area B). We considered that neutropenia could be decreased with patients in area A by individualized dosing. However, it is unknown whether the therapeutic effect of docetaxel could be improved in the patients in area B by individualized dosing because no significant positive correlation has been found between docetaxel AUC and antitumor response in patients with non–small-cell lung cancer.[43] In this study, seven of 30 (23.3%) and two of 30 (6.7%) patients in the BSA-based arm were included in area A and B, respectively (Figs 3 and 5).

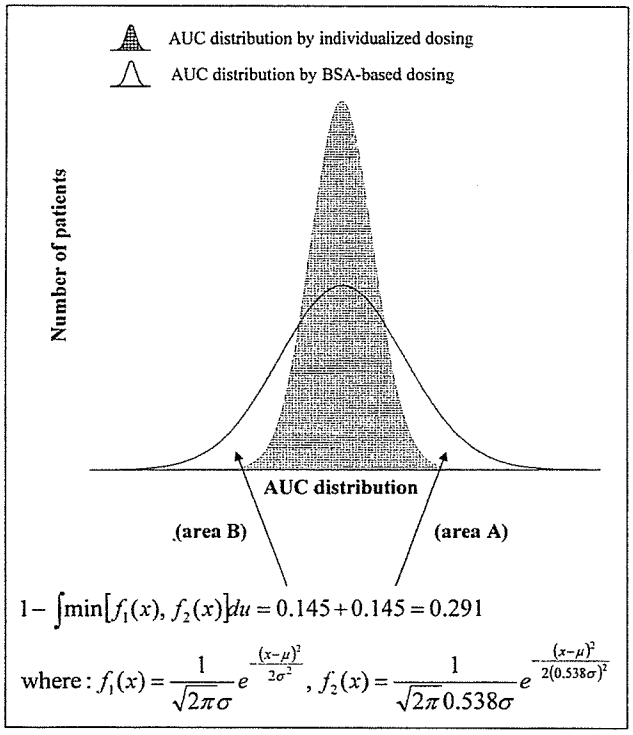

Fig 5. Simulated comparison of area under the concentration-time curve (AUC) distribution between body-surface area (BSA) –based dosing and individualized dosing when the variability of AUC is decreased 46.2% by individualized dosing applied using our method.

As shown in Figure 4, the percentage decrease in ANC was well correlated with AUC in both arms, which was similar to previous reports.[37,43] It was also indicated that the interpatient variability in the percentage decrease in ANC was slightly smaller in the individualized arm than in the BSA-based arm; however, this difference was not significant. The response rates between the two arms were similar. Although the interpatient PK variability could be decreased by individualized dosing in accordance with our method, the interpatient PD variability such as toxicity and the antitumor response could not be decreased. Several reasons could be considered.

With regard to toxicity, the pretreatment characteristics of the patients in this study were highly variable. More than half of the patients in each arm had previously received platinum-based chemotherapy, and more than 30% had received radiotherapy. The laboratory parameters (ie, ALB, AAG, and ALP) were not balanced across the arms, although they were not included in the eligibility criteria (Table 1). These variable pretreatment characteristics and unbalanced laboratory parameters may have influenced the frequency and severity of the hematologic toxicity as well as the pharmacokinetic profiles. The antitumor effect may have been influenced by the intrinsic sensitivity of tumors, the variable pretreatment characteristics, and the imbalance in laboratory parameters. Non–small-cell lung cancer is a chemotherapy-resistant tumor. The response rate for docetaxel ranges from 18% to 38%,[5] and no significant positive correlation between docetaxel AUC and antitumor response has been found. We considered it quite difficult to control the interpatient PD variability by controlling the interpatient PK variability alone. Although we did not observe any outliers in either arm, such as the two outliers with severe toxicity observed in the study by Hirth et al,[28] our method may be more useful for identifying such outliers. If we had not excluded patients with more abnormal liver function or a history of liver disease by the strict eligibility criteria, the results with the two dosing regimens may have been more different, and the interpatient PD variability, such as the percentage decrease in ANC, may have been smaller in the individualized arm than in the BSA-based arm. Furthermore, the primary end point of this study was PK variability, evaluated by the SD of AUC in both arms, and the sample size was significantly underpowered to evaluate whether the application of our method to individualized dosing could decrease PD variability compared with BSA-based dosing.

For the genotypes of CYP3A4, several genetic polymorphisms have been reported (http://www.imm.ki.se/CYPalleles/); however, a clear relationship between genetic polymorphisms and the enzyme activity of CYP3A4 has not been reported. Our phenotype-based

individualized dosing using the total amount of urinary 6-β-OHF after cortisol administration produced good results. However, this method is somewhat complicated, and a simpler method would be of great use. We analyzed the expression of CYP3A4 mRNA in the peripheral-blood mononuclear cells of the 29 patients in the individualized arm. No correlation was observed between the expression level of CYP3A4 mRNA and docetaxel CL or the total amount of urinary 6-β-OHF after cortisol administration (data not shown).

In conclusion, the individualized dosing of docetaxel using the total amount of urinary 6-β-OHF after cortisol administration is useful for decreasing the interpatient PK variability compared with the conventional BSA-based method of dosing. This method may be useful for individualized chemotherapy.

Authors' Disclosures of Potential Conflicts of Interest

The authors indicated no potential conflicts of interest.

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Lung Cancer (2005) 49, 217–223

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Clinical responses of large cell neuroendocrine carcinoma of the lung to cisplatin-based chemotherapy

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Received 30 September 2004; received in revised form 3 January 2005; accepted 3 January 2005

KEYWORDS
Neuroendocrine carcinoma;
Lung cancer;
Chemotherapy;
Cisplatin

Summary
Background: The efficacy of chemotherapy in patients with large cell neuroendocrine carcinoma of the lung (LCNEC) remains unclear.
Methods: Patients with LCNEC who received cisplatin-based chemotherapy were identified by reviewing 567 autopsied and 2790 surgically resected lung cancer patients. The clinical characteristics and objective responses to chemotherapy in these patients were analyzed.
Results: Overall, 20 cases of LCNEC were identified, including stage IIIA ($n=3$), stage IIIB ($n=6$), stage IV ($n=6$) and postoperative recurrence ($n=5$) cases. Six patients had received prior chemotherapy, and 14 were chemo-naive patients. The patients had received a combination of cisplatin and etoposide ($n=9$), cisplatin, vindesine and mitomycin ($n=6$), cisplatin and vindesine ($n=4$), or cisplatin alone ($n=1$). One patient showed complete response and nine showed partial response, yielding an objective response rate of 50%. The response rate did not differ between patients with the initial diagnosis of SCLC and those with the initial diagnosis of NSCLC, however, the response rate in chemo-naive patients (64%) was significantly different from that in previously treated patients (17%).
Conclusions: Our results suggest that the response rate of LCNEC to cisplatin-based chemotherapy was comparable to that of SCLC.

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doi:10.1016/j.lungcan.2005.01.008

1. Introduction

Pulmonary neuroendocrine tumors include a spectrum of four clinicopathological entities classified on the basis of the morphological and biological features: typical carcionoid and atypical carcinoid, which are tumors of low to intermediate grade malignancy, and large cell neuroendocrine carcinoma (LCNEC) and small cell carcinoma (SCLC), which are high-grade malignant tumors. Travis et al. proposed the term LCNEC in 1991 [1], for classifying a type of poorly differentiated high-grade carcinoma characterized by a neuroendocrine appearance under light microscopy. LCNEC exhibits more prominent cellular pleomorphism and higher mitotic activity than the atypical carcinoid (AC), and is distinguished from SCLC by the tumor cell size and chromatin morphology. Although several different terminologies and classifications have been proposed previously, and even the present classification of pulmonary neuroendocrine tumors lacks uniform definition criteria, this class of tumors could become widely accepted and included in the updated histological classification of the World Health Organization [2].

The clinical features of LCNEC have not yet been completely clarified. The prognosis of patients with surgically resected LCNEC is reported to be intermediate between that of AC and SCLC [3–5], and the same as that of resected NSCLC, except that stage I LCNEC has a poorer prognosis than stage I non-small cell lung cancer (NSCLC) [6]. To the best of our knowledge, however, there are no studies that have examined the role of chemotherapy for LCNEC and the prognosis of patients with unresectable LCNEC, even though several reports have been published on the association between response to chemotherapy and the neuroendocrine differentiation of NSCLC [7–9]. The appropriate treatment of unresectable LCNEC, therefore, remains unclear. In the present study, we attempted to investigate the effectiveness of chemotherapy with cisplatin-based regimens for LCNEC in patients with unresectable and recurrent LCNEC.

2. Materials and methods

Eighty-seven of 2790 patients with primary lung cancer who underwent tumor resection from 1982 to 1999 at the National Cancer Center Hospital were found to have tumors with the histological characteristics of LCNEC [6]. Of these, five had received cisplatin-based chemotherapy at the time of recurrence, and were enrolled as subjects of this study. In addition, 303 of 567 patients who were autopsied from 1983 to 1997 at the National Cancer Center Hospital who had the following histological diagnoses were first selected: SCLC ($n=112$), poorly differentiated adenocarcinoma ($n=99$), large cell carcinoma ($n=58$), poorly differentiated squamous cell carcinoma ($n=29$), poorly differentiated adenosquamous carcinoma ($n=2$), LCNEC ($n=2$), and carcinoid ($n=1$). Of these, 161 had received cisplatin-based chemotherapy were selected for a pathological review. Finally, specimens from 17 of these161 cases were found to have histological characteristics consistent with the diagnosis of LCNEC, and were selected as subjects of this study. We focused on cisplatin, because since the 1980s, cisplatin has been the only anticancer agent with proven efficacy against both SCLC and NSCLC [10,11]; we, therefore, considered that the effectiveness of chemotherapy for LCNEC could be reasonably evaluated if cisplatin were included in the regimen. Cases which had received adjuvant chemotherapy without evaluable lesions were excluded from the analysis.

All the available paraffin-embedded tissue sections stained with hematoxylin–eosin were reviewed. We classified LCNEC according to the histopathological criteria in the WHO classification [2]. Immunohistochemical analysis was performed to confirm the neuroendocrine features of the tumors. For this purpose, formalin-fixed paraffin sections were stained for a panel of neuroendocrine markers, including chromogranin A (CGA), synaptophysin (SYN), and neural cell adhesion molecule (NCAM), using standard methods. The intensity of immunostaining for these markers was scored as follows: +, when the proportion of stained tumor cells was >50%; ±, when 10–50% of tumor cells were stained; and −, when <10% of tumor cells were stained, as previously described [6]. One case included in this study had the typical histological features of LCNEC, but no neuroendocrine features as determined by the immunohistochemical analysis. For specimens obtained after treatment, we routinely confirmed that the histopathological and morphological features showed no changes due to treatment as compared with the pretreatment biopsy or cytologic specimens. Such cases for which no pretreatment samples were available were excluded from the study; since it has been reported that histological changes may occur after treatment in SCLC [12], we were concerned that misdiagnosis might occur if the same were also true for LCNEC.

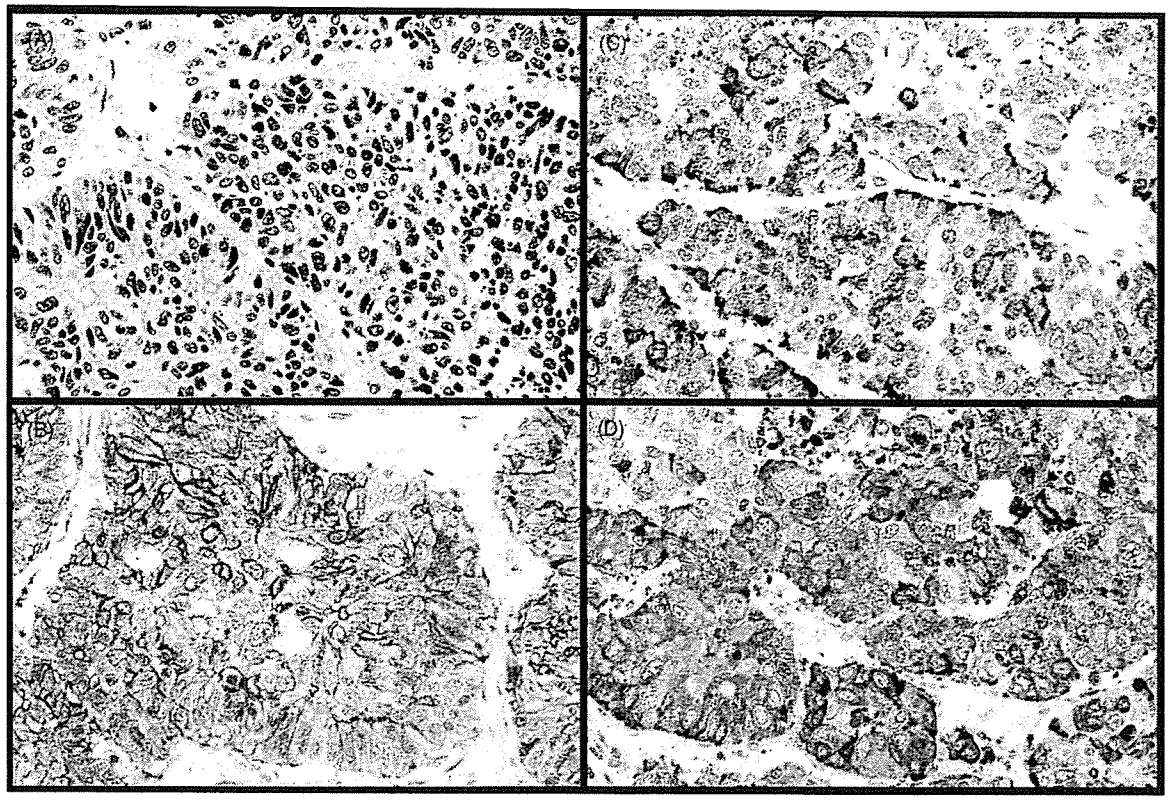

Fig. 1 Case no. 2, 57-year-old man. (A) The tumor cells which are large-sized, polygonal in shape and have a low nuclear-cytoplasmic ratio, are arranged in organoid nests and trabeculae (H&E stain, ×200). Positive staining for neural cell adhesion molecule (B), chromogranin A (C), and synaptophysin (D) (immunostain, ×400).

Clinical information about the cases was obtained from the medical records. The clinical disease staging was reassessed according to the latest International Union Against Cancer (UICC) staging criteria [13]. The response to chemotherapy and overall survival rate were assessed retrospectively. The objective tumor response was evaluated according to the WHO criteria published in 1979 (WHO, 1979) [14]. The survival time was measured from the date of start of chemotherapy with a cisplatin-containing regimen. Survival curves were drawn using the Kaplan–Meier method [15]. Drug toxicity could not be assessed as the study was a retrospective one and records were often incomplete.

3. Results

Overall, 22 cases were recognized as having tumors with histological characteristics consistent with LCNEC among the autopsied and surgically resected cases of primary lung cancer that had received cisplatin-based chemotherapy and had evaluable lesions; of these 17 were autopsied cases and five were surgically resected cases. Two of the autopsied cases were excluded, because no pretreatment pathological or cytological samples were available. The typical microscopic appearance of the tumor specimens is shown in Fig. 1A. The specimen sources for the prechemotherapy-diagnosis included surgically resected specimens ($n=5$), biopsy specimens ($n=9$), and cytology specimens ($n=6$). The histological and cytological findings in the specimens obtained before chemotherapy were consistent with those in the specimens obtained after chemotherapy. We therefore finally enrolled 20 cases in this study. The initial pathologic diagnoses in these patients were as follows: small cell carcinoma ($n=10$), poorly differentiated adenocarcinoma ($n=6$), large cell carcinoma ($n=2$), undifferentiated carcinoma ($n=1$), and poorly differentiated carcinoma ($n=1$) (Table 1). None of the cases had been labeled as LCNEC at the time of initial diagnosis, probably because the concept of LCNEC

Table 1 Patient characteristics

Characteristics	N	%
No. of patients	20	
Sex		
Male	18	90
Female	2	10
Age, median (range)	58 (37–74)	
Smoking history		
Yes	19	95
No	1	5
Performance status		
1–2	19	95
>2	1	5
Initial pathological diagnosis		
Small cell carcinoma	10	50
Adenocarcinoma	6	30
Large cell carcinoma	2	10
Others	2	10
Clinical stage at the start of chemotherapy		
IIIA	3	15
IIIB	6	30
IV	6	30
Postoperative recurrence	5	25
Prior treatment		
None	10	50
Surgery	4	20
Radiotherapy	2	10
Chemotherapy without cisplatin	6	30

Table 2 Staining for neuroendocrine markers in 20 LCNECs

Case	NCAM	CGA	SYN
1	+	+	+
2	+	+	+
3	+	+	+
4	±	+	+
5	+	+	+
6	+	+	+
7	−	+	−
8	+	−	−
9	−	−	−
10	−	+	±
11	+	−	+
12	+	+	+
13	+	+	+
14	+	−	±
15	+	+	+
16	+	−	NA
17	+	−	+
18	+	−	NA
19	+	−	+
20	−	+	+

NCAM, neural cell adhesion molecule; CGA, chromogranin A; SYN, synaptophysin; NA, not assessed.

was not completely accepted at our hospital at that time.

The results of the immunohistochemical staining are shown in Table 2, and a typical case showing positive staining is shown in Fig. 1B and D. Of the 20 LCNECs, 19 expressed at least one of the three general neuroendocrine markers, namely CGA, SYN, and NCAM. Sixteen of the 20 LCNECs exhibited positive staining for NCAM, while one showed equivocal staining. Twelve of the 20 LCNECs showed positive staining for CGA. Thirteen LCNECs showed positive staining for SYN and three showed equivocal staining. Only one case was negative for all the three general neuroendocrine markers, however, this case exhibited the typical histological features of LCNEC on light microscopy.

The clinical characteristics of the patients are summarized in Table 1. The extremely high predominance of men and smokers in this study was comparable to the demographic features of our LCNEC patients treated by surgical resection [6]. Previous chemotherapy was given in six patients: nedaplatin in one and cyclophosphamide-based regimen in five patients. The chemotherapy regimens used were as follows: cisplatin (80 mg/m^2, day 1) and etoposide (100 mg/m^2, days 1–3) (n = 9), cisplatin (80 mg/m^2, day 1), vindesine (3 mg/m^2, days 1 and 8) and mitomycin (8 mg/m^2, day 1) (n = 6), cisplatin (80 mg/m^2, day 1) and vindesine (3 mg/m^2, days 1 and 8) (n = 4), or cisplatin (100 mg/m^2, day 1) alone (n = 1). The median (range) number chemotherapy cycles were 2 (1–6). Of the 20 patients, one showed CR and nine showed PR, yielding an overall response rate of 50% (95% confidence interval, 27.2–72.8%). One CR and four PRs were observed among the cases treated with cisplatin and etoposide, two PRs were found among those treated with cisplatin, vindesine and mitomycin, and three PRs were found among those treated with cisplatin and vindesine. Seven patients showed NC, and three showed progressive disease. While the response rate did not differ between patients with an initial diagnosis of SCLC and those patients with an initial diagnosis of NSCLC, previous chemotherapy affected the response to cisplatin: the response rate in chemo-naive patients was 64%, whereas that in previously treated patients was 17%. The median progression-free survival in the 20 patients was 103 days, median survival was 239 days, 1-year survival rate was 35%, and 2-year survival rate was 15%.

4. Discussion

In this extensive review of over 3000 lung cancer patients, we found considerable difficulty in evaluating the response of LCNEC to systemic chemotherapy. The pathological diagnosis of LCNEC was established in 87 (3.1%) of 2790 patients treated by surgical resection. This low incidence of LCNEC in surgically treated lung cancer patients is comparable to that in other previously published reports: 2.4% (50/2070), 2.9% (22/766), and 3.6% (53/1530) [16–18]. Of the 87 patients, only five who had received cisplatin-based chemotherapy for recurrent tumor that was evaluable for the response. While LCNEC is difficult to diagnose prior to the start of treatment on the basis of the findings in biopsy or cytological specimens, the architectural neuroendocrine features may, more or less, be reflected in these small samples [19,20]. We, therefore, conducted a review of 567 autopsy cases of lung cancer, and identified 15 cases of LCNEC who had received cisplatin-based chemotherapy. We obtained a response rate to cisplatin-based chemotherapy of 50% in these 20 patients with LCNEC, however, the clinical characteristics of patients with medically treatable advanced LCNEC would still remain to be clarified, because autopsy is conducted only in highly selective cases.

Travis et al. suggested that immunohistochemical or electron-microscopic evidence of neuroendocrine features were important to diagnose LCNEC [1]. We assessed the neuroendocrine marker expression by immunohistochemical staining for CGA, SYN, and NCAM. Our cases included one that was negative for all the three neuroendocrine markers examined, but showed the typical histological features of LCNEC, which could be attributable to technical staining problems. Immunohistochemical staining for neuroendocrine tumors is generally recognized as only a supplementary diagnostic tool. In addition, the post-surgical survival rate did not differ between histologically diagnosed cases of LCNEC with neuroendocrine differentiation in marker expression as assessed by immunohistochemical staining and large cell carcinoma with neuroendocrine morphology where the neuroendocrine markers were negative (data not shown). Thus, we decided to include the case with negative staining as LCNEC on the basis of its typical neuroendocrine morphology.

To the best of our knowledge, only one study on the efficacy of chemotherapy in patients with LCNEC has been reported previously. In the study, 13 patients with LCNEC received chemotherapy when relapse was noted after surgical resection, and two (20%) of 10 evaluable patients showed an objective response. The evaluable lesion in these patients, however, was the brain in seven, liver in two, and bone in one patient [21]. Thus, the relatively low response rate in the report may be due to the site of the evaluable lesion. In addition, reports on the correlation between response to chemotherapy and neuroendocrine differentiation of NSCLC may be helpful. Graziano et al. reported that the proportion of NSCLC positive for neuroendocrine markers was higher in responders than in non-responders among 52 NSCLC patients treated by chemotherapy, and that the result suggested a correlation between positivity for neuroendocrine marker expression and the likelihood of response to chemotherapy [7]. On the other hand, others have reported the absence of any correlation between the presence of neuroendocrine differentiation and the response to chemotherapy [8,9]. The neuroendocrine differentiation in NSCLCs in the aforementioned studies was confirmed only by immunohistochemical staining and not on the basis of the morphological definition of LCNEC. Therefore, these groups might have potentially included heterogeneous subtypes of lung carcinoma, such as adenocarcinoma or squamous cell carcinoma, with components of neuroendocrine differentiation. The conflicting conclusions of these studies may, therefore, reflect differences in the biological characteristics of the tumors included in the analysis. Since the definition of LCNEC is based on morphological criteria as well as positivity for neuroendocrine marker expression, LCNEC is may be considered to be a clinically homogeneous group. Therefore, our study of LCNEC may endorse the former reports about the relationship between neuroendocrine differentiation and the sensitivity to chemotherapy.

Objective response to chemotherapy can be observed in only 15–30% of NSCLCs, even when they are treated with regimens containing cisplatin [10]. In SCLC, however, effective combination regimens yield objective response rates in the range of 80–90% [11]. Our study showed an overall response rate of LCNEC of 50% to cisplatin-based chemotherapy, and a response rate of 64% in chemo-naive patients, which seemed to be higher than the response rate of NSCLC to chemotherapy. Considered together, these results suggest that the chemosensitivity of LCNEC is intermediate between that of NSCLC and SCLC, although we were unable to obtain firm evidence from this retrospective study, which included only a small cohort of patients.

Since LCNEC is a relatively rare subtype of lung cancer, a prospective study is difficult to perform, and may only be possible as a multicenter study.

For this purpose, it is an urgent task to establish diagnostic criteria for LCNEC based on examination of biopsy or cytologic specimens. Although the histological definition of LCNEC in surgically resected specimens proposed by Travis et al. is commonly accepted, its diagnostic reproducibility is not satisfactory [22]. It is also difficult to apply the definition to biopsy specimens, in which artifacts can easily be produced and detailed examination may be difficult due to insufficient specimen size. Thus, definitive diagnostic criteria also applicable to biopsy and cytologic specimens are required.

Our study did not include any cases labeled as LCNEC at the time of initial diagnosis. One half of the cases was originally diagnosed as SCLC and the other half as NSCLC, including poorly differentiated adenocarcinoma and large cell carcinoma. This was attributed to the fact that the concept of LCNEC was not clearly defined prior to its being proposed by Travis et al. [1]. Thus, it is possible that patients with LCNEC were included in earlier clinical trials for NSCLC or SCLC. If LCNEC shares the poor prognosis of NSCLC, the reported results of chemotherapy for NSCLC may have been worse in studies in which cases of LCNEC were included. Similarly, the results of clinical studies of SCLC to study their objective response to chemotherapy may also have been worse because of the confounding effects of the inclusion of LCNECs among the cases.

In conclusion, our results suggest that the response rate of LCNEC to cisplatin-based chemotherapy was comparable to that of SCLC. However, because of the retrospective nature of this study and the small sample size, we could not arrive at any definitive conclusion; we, therefore, propose to conduct a prospective study in the future aimed at elucidating the efficacy of chemotherapy for LCNEC. To that end, firm diagnostic criteria for LCNEC need to be established, even when the diagnosis must be based only on examination of biopsy and cytology specimens.

Acknowledgment

We thank Ms. Yuko Yabe for kindly preparing this manuscript.

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Lung Cancer (2004) 45, 93–104

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Risk factors for interstitial lung disease and predictive factors for tumor response in patients with advanced non-small cell lung cancer treated with gefitinib

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Received 22 September 2003; received in revised form 2 January 2004; accepted 9 January 2004

KEYWORDS
Gefitinib;
Non-small cell lung cancer;
Interstitial lung diseases;
Pulmonary fibrosis;
Risk factors;
Predictive factors

Summary A high incidence of interstitial lung disease (ILD) has been reported in patients with non-small cell lung cancer (NSCLC) treated with gefitinib in Japan. We retrospectively analyzed 112 patients with advanced NSCLC who received gefitinib monotherapy. Univariate and multivariate analyses were used to identify risk factors for gefitinib-related ILD and predictive factors for tumor response to gefitinib. The incidence of ILD was 5.4%, and it was higher in the patients with pre-existing pulmonary fibrosis (33% versus 2%; $P < 0.001$). The results of a multivariate analysis showed that pulmonary fibrosis was a significant risk factor for ILD (odds ratio: 177, 95% confidence interval: 4.53–6927, $P = 0.006$). The response rate was 33% in the 98 evaluable patients and higher in women (53% versus 23%; $P = 0.003$), patients with adenocarcinoma (38% versus 6%; $P = 0.010$), never-smokers (63% versus 18%; $P < 0.001$), and the patients with no history of thoracic radiotherapy (39% versus 13%; $P = 0.015$). The results of a multivariate analysis showed that the predictors of tumor response were "no history of smoking" and "no history of thoracic radiotherapy". Never-smokers had a significantly longer survival time than smokers ($P = 0.007$). Although gefitinib therapy confers a clinical benefit on patients with advanced NSCLC, especially on women, patients with adenocarcinoma, never-smokers, and patients with no history of thoracic radiotherapy, it also poses a high risk of ILD, especially to patients with pulmonary fibrosis. The risk-benefit ratio must be carefully considered.

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0169-5002/$ - see front matter © 2004 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.lungcan.2004.01.010

1. Introduction

Gefitinib (Iressa®; AstraZeneca, Osaka, Japan) is an orally available, selective epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor that displays antitumor activity in patients with previously treated advanced non-small cell lung cancer (NSCLC). The safety and tolerability of gefitinib was established in four open-labeled, multicenter, phase I dose-escalation studies [1–4]. Although diarrhea, skin rash/acne, and nausea were common adverse effects, most of them were mild. Two large-scale, multicenter, randomized phase II studies (IDEAL 1 and 2; Iressa® Dose Evaluation in Advanced Lung Cancer) have demonstrated clinically significant antitumor activity of gefitinib monotherapy in patients with advanced NSCLC who had previously received platinum-based chemotherapy [5,6]. The response rate for gefitinib 250 mg per day in the IDEAL 1 and 2 trials was 18.4 and 11.8%, respectively. These studies also showed that gefitinib monotherapy significantly improved disease-related symptoms and quality of life.

Based on the results of the IDEAL trials, gefitinib was approved in Japan for the treatment of inoperable or recurrent NSCLC on 5 July 2002, and an estimated 28,300 patients had been treated with gefitinib as of April 2003. During the first few months after its approval, many patients demanded to be treated with gefitinib as a "magic bullet" cure; however, when the incidence of interstitial lung disease (ILD) came to light in October 2002, the media reported it in a sensational manner, and as a result patients have become confused by excessive expectations and fear of ILD. The Ministry of Health, Labour and Welfare of Japan reported that the number of gefitinib-related cases of ILD had reached 616 as of 22 April 2003 and that 246 of the patients had died of it. The incidence of ILD and mortality rate from it has been calculated at 2.2 and 0.87%, respectively. Some case reports also suggested a high incidence of gefitinib-related ILD in Japan [7]. In view of this situation, an evidence-based assessment of the risk-benefit of gefitinib for the treatment of NSCLC was urgently needed. However, many questions regarding gefitinib administration remained unanswered, particularly in regard to the risk factors associated with ILD complications. We therefore analyzed a series of cases treated with gefitinib at the National Cancer Center Hospital (NCCH) in Tokyo.

2. Patients and methods

Between July and December 2002, 115 NSCLC patients at the NCCH began taking gefitinib and the 112 of these patients who were followed at the NCCH were retrospectively analyzed in this study. The other three patients were excluded from the analysis because they were followed-up at other hospitals after the first prescription of gefitinib. All the 112 patients had histologically or cytologically confirmed NSCLC. Their disease was locally advanced, recurrent, and/or metastatic. They all received gefitinib monotherapy at a dose of 250 mg per day.

Two independent board-certified diagnostic radiologists (M.K. and U.T.) diagnosed pre-existing pulmonary fibrosis (PF) on the basis of the findings on chest X-rays taken within 1 week of the start of gefitinib therapy. The radiologists had no knowledge of the patients' outcome. The diagnostic criteria for PF were a diffuse linear or honey-comb pattern on chest X-rays that was predominant in the lower zone of the lung.

If a patient had measurable disease, the World Health Organization criteria were used to assess the tumor response. The response rate was calculated as the total percentage of patients with a complete or partial response. Drug-related adverse events were evaluated using the National Cancer Institute-Common Toxicity Criteria (Version 2.0). Chest X-rays were performed periodically to evaluate response and detect pulmonary toxicity, and computed tomography scans of the chest were performed as needed to confirm the response or diagnose ILD. The extent of patients' smoking history was evaluated by using pack-years, which are defined as the average number of cigarettes smoked per day multiplied by the total duration of smoking in years divided by 20. Patients who had smoked for 0, 1–39, and $\geq$40 pack-years were categorized as "never-smokers", "moderate smokers", and "heavy smokers", respectively.

Univariate and multivariate analyses were performed to identify risk factors for ILD and predictive factors for tumor response to gefitinib. The patient characteristics tested as potential risk factors for ILD and predictive factors for tumor response were age ($<$70 versus $\geq$70 years in the univariate analysis and as a continuous variable in the multivariate analysis), sex (female versus male), histological diagnosis (adenocarcinoma versus non-adenocarcinoma), smoking history (never-smokers versus moderate/heavy smokers), performance status (PS 0–1 versus PS 2–3), prior surgery (yes versus no), prior chemotherapy (yes versus no), prior thoracic radiotherapy (yes versus no), and PF (yes versus no). These factors were compared by using a chi-square test in the univariate analysis. Logistic regression analyses were also performed to adjust for each factor. Differences

in time to treatment failure (TTF) and overall survival (OS) among the subgroups were compared by using Kaplan–Meier curves and log-rank tests. TTF was defined as the interval between the start of gefitinib administration and discontinuation of treatment for any reason, confirmed disease progression, or death. All analyses were performed using SPSS statistical package (SPSS version 11.0 for Windows, SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Patient characteristics

The patient characteristics are listed in Table 1. All patients were Japanese. Twenty-eight patients (25%) received gefitinib as a first-line treatment; 19 were considered unfit for platinum-based chemotherapy because of poor PS (10 patients) or advanced age (9 patients), and 9 refused platinum-based chemotherapy. The diagnosis of pre-existing PF was almost the same between two radiologists. Although discordance occurred in three cases, 12 patients were finally diagnosed as PF by consensus. All of the 12 patients had computed tomography findings consistent with idiopathic pulmonary fibrosis/usual interstitial pneumonia.

3.2. Interstitial lung disease (ILD) and other toxicities

Among the 112 patients reviewed, ILD developed in 6 (5.4%) during the course of gefitinib therapy, and 4 patients (3.6%) died from ILD. The characteristics of the six patients with ILD are listed in Table 2. All of them had acute onset or exacerbation of respiratory symptoms. In five patients, chest computed tomography scanning revealed new diffuse interstitial changes in both lungs with ground-glass appearances. Because bronchoalveolar lavage or lung biopsy was not performed, we cannot completely exclude lymphangiosis carcinomatosa or other diseases, but the clinical courses and imaging appearances were consistent with drug-induced ILD. Although the other patient (patient 3) died before imaging diagnosis, the autopsy revealed diffuse alveolar damage, and we concluded she died from gefitinib-related ILD.

The results of univariate and multivariate analyses on risk factors for ILD are shown in Table 3. The incidence of ILD was 33% (4/12) among patients with PF and 2.0% (2/100) among the other patients. PF was the only significant risk factor for ILD in the univariate analysis (odds ratio [OR]: 16.7, 95% confidence interval [95% CI]: 3.40–83.3, $P < 0.001$), and this finding was supported by the results of the multivariate analysis (OR: 177, 95% CI: 4.53–6927, $P = 0.006$). Since all of the patients with ILD were smokers, pack-years were analyzed as a continuous variable in the multivariate analysis, and the results of it suggested the association between increased pack-years and a higher risk of ILD ($P = 0.062$). Since all of the ILD cases had a PS score of 1 and had never undergone thoracic radiotherapy, it was impossible to assess the association between poor PS or prior thoracic radiotherapy and ILD in the multivariate analysis.

Table 1 Patient characteristics

	Patients ($n = 112$)	
	No.	%
Age		
Median (range) (years)	63 (29–83)	
<70 years	80	71
≥70 years	32	29
Sex		
Female	35	31
Male	77	69
Histological diagnosis		
Adenocarcinoma	93	83
Squamous cell carcinoma	12	11
Non-small cell carcinoma (not specified)	6	5
Large cell neuroendocrine carcinoma	1	1
Smoking history (pack-years)		
Never-smokers (0)	34	30
Moderate smokers (1–39)	30	27
Heavy smokers (≥40)	48	43
ECOG performance status		
0–1	92	82
2–3	20	18
Stage		
IIIA/IIIB	21	19
IV	58	52
Recurrence after surgery	33	29
Prior chemotherapy		
Yes	84	75
No	28	25
Prior thoracic radiotherapy		
Yes	26	23
No	86	77
Pre-existing pulmonary fibrosis		
Yes	12	11
No	100	89

Table 2 Characteristics of patients who developed interstitial lung disease

	Age (years)	Sex	Histological diagnosis	PS	PY	Stage	Prior chemotherapy		Thoracic radiotherapy	Pre-existing lung disease	Length of treatment (days)	Survival (days)
							First	Second				
1	66	M	Ad	1	44	IIIB	CDDP+VNR	DTX	No	PF	10	22[a]
2	69	M	Ad	1	28	IV	CBDCA+PTX	–	No	PF	32	67[a]
3	52	F	Ad	1	48	IV	CDDP+GEM	–	No	None	42	42[a]
4	71	M	Ad	1	51	IIIB	UFT	–	No	PF	47	123[a]
5	64	M	Sq	1	129	IV	CBDCA+PTX	DTX	No	None	18	237[b]
6	74	M	Ad	1	64	Rec	CBDCA+PTX	–	No	PF	39	400[b]

Ad: adenocarcinoma, Sq: squamous cell carcinoma, PS: performance status, PY: pack-years smoked, Rec: recurrence after surgery, CDDP: cisplatin, CBDCA: carboplatin, VNR: vinorelbine, DTX: docetaxel, PTX: paclitaxel, GEM: gemcitabine, PF: pulmonary fibrosis.

[a] Treatment-related death.

[b] Death from lung cancer.

Table 3 Risk factors for interstitial lung disease ($n = 112$)

	No. of patients	Incidence of ILD (%)	Univariate analysis		Multivariate analysis	
			Odds ratio (95% CI)	*P*-values	Odds ratio (95% CI)	*P*-values
Total	112	5.4				
Age						
<70 years	80	5.0	0.80 (0.15–4.18)	0.791	2.05 (0.46–9.17)	0.347[a]
≥70 years	32	6.3	1			
Sex						
Female	35	2.9	0.44 (0.053–3.62)	0.428	19.1 (0.44–837)	0.126
Male	77	6.5	1		1	
Histological diagnosis						
Adenocarcinoma	93	5.4	1.02 (0.13–8.26)	0.984	0.26 (0.012–5.46)	0.383
Non-adenocarcinoma	19	5.3	1		1	
Smoking history (pack-years)						
Heavy smokers (≥40)	48	10.4	–	0.096[b]	1.50 (0.98–2.29)	0.062[c]
Moderate smokers (1–39)	30	3.3	–			
Never-smokers (0)	34	0.0	1			
PS						
2–3	20	0.0	0	0.240		
0–1	92	6.5	1			
Prior surgery						
Yes (recurrence)	33	3.0	0.48 (0.056–3.94)	0.480	2.48 (0.14–43.2)	0.534
No (advanced disease)	79	6.3	1		1	
Prior chemotherapy						
Yes	84	7.1	–	0.146		
No	28	0.0	1			
Prior thoracic radiotherapy						
Yes	26	0.0	0	0.166		
No	86	7.0	1			
Pulmonary fibrosis						
Yes	12	33	16.7 (3.40–83.3)	<0.001	177 (4.53–6927)	0.006
No	100	2.0	1		1	

CI: confidence interval.
[a] Age was analyzed as a continuous variable in the multivariate analysis. Odds ratio was calculated per 10-year decrease.
[b] Smoking history was analyzed by comparing never-smokers and moderate/heavy smokers in the univariate analysis.
[c] Smoking history (pack-years) was analyzed as a continuous variable in the multivariate analysis. Odds ratio was calculated per 10-pack-year increase.

The incidence of drug-related adverse events is listed in Table 4. Grade 1 or 2 skin rash (81%) and diarrhea (56%) were the most frequent adverse events. Grades 1–3 elevation in glutamic-oxaloacetic transaminase (GOT) and/or glutamic-pyruvic transaminase (GPT) levels was observed in 46% of the patients.

3.3. Efficacy

Of the 112 patients, 98 had measurable disease. Four patients were not evaluated due to early discontinuation. Complete response, partial response, stable disease, and progressive disease were observed in 2, 30, 29, and 33 patients,

Table 4 Toxicity

	No. of patients evaluated	Grade			
		1	2	3	4
Skin rash	109	59	29	0	0
Diarrhea	109	57	4	0	0
GOT/GPT	106	31	8	10	0
Nausea	109	21	5	0	0
Interstitial lung disease (ILD)	112	0	1	1	4[a]

[a] Treatment-related death.

respectively. The response rate was 33% (32/98). The response rates in each subgroup of patients are listed in Table 5. According to the results of the univariate analysis, female gender ($P = 0.003$), adenocarcinoma ($P = 0.010$), no history of smoking ($P < 0.001$), and no history of thoracic radiotherapy ($P = 0.015$) were significant predictors of tumor response to gefitinib. The response rate of male smokers was 14% (8/56), which was lower than both that of female smokers (40%, $P = 0.052$) and that of male never-smokers (70%, $P < 0.001$). When pack-years were analyzed as a continuous variable among the smokers, the association between

Table 5 Response rates among subgroups of patients ($n = 98$)

	No. of patients	Response rate (%)	Univariate analysis		Multivariate analysis	
			Odds ratio (95% CI)	*P*-values	Odds ratio (95% CI)	P values
Total	98	33				
Age						
<70 years	69	36	1.50 (0.76–2.97)	0.244	1.57 (0.96–2.56)	0.071[a]
≥70 years	29	24	1			
Sex						
Female	32	53	2.34 (1.34–4.06)	0.003	1.84 (0.51–6.56)	0.349
Male	66	23	1		1	
Histological diagnosis						
Adenocarcinoma	81	38	6.51 (1.58–26.8)	0.010	4.27 (0.48–37.0)	0.191
Non-adenocarcinoma	17	6	1		1	
Smoking history (pack-years)						
Never-smokers (0)	32	63	3.44 (1.98–5.97)	<0.001[b]	3.92 (1.03–14.9)	0.045[b]
Moderate smokers (1–49)	22	23	1		1	
Heavy smokers (≥50)	44	16				
PS						
0–1	83	31	0.78 (0.38–1.62)	0.510	0.46 (0.10–2.09)	0.314
2–3	15	40	1		1	
Prior surgery						
No (advanced disease)	68	28	0.64 (0.36–1.14)	0.134	1.25 (0.35–4.41)	0.732
Yes (recurrence)	30	43	1		1	
Prior chemotherapy						
No	24	42	1.40 (0.76–2.58)	0.279	1.32 (0.35–4.95)	0.678
Yes	74	30	1		1	
Prior thoracic radiotherapy						
No	74	39	3.14 (1.24–7.90)	0.015	6.76 (1.30–35.7)	0.023
Yes	24	13	1		1	

CI: confidence interval.

[a] Age was analyzed as a continuous variable in the multivariate analysis. The odds ratio was calculated per 10-year decrease.

[b] Smoking history was analyzed by comparing never-smokers and moderate/heavy smokers.

increased pack-years and a lower response rate was also shown (OR per 10-pack-year increase: 0.74, 95% CI: 0.56–0.99, $P = 0.041$).

The results of a multivariate analysis showed that "no history of smoking" ($P = 0.045$) and "no history of thoracic radiotherapy" ($P = 0.023$) were significant predictors of response. It was also suggested that younger patients tended to obtain a higher response rate ($P = 0.071$). Although female gender and adenocarcinoma were not found to be predictive factors in the multivariate analysis, sex and histological diagnosis were significantly associated with smoking history, and these variables may have canceled each other's effect on the dependent variable. The proportion of never-smokers was 69% (22/32) among the women versus 15% (10/66) among the men (correlation coefficient [r] = 0.536, $P < 0.001$), and 67% (54/81) among the patients with adenocarcinoma versus 0% (0/17) among those with non-adenocarcinoma ($r = 0.319$, $P = 0.001$). When a multivariate analysis was performed excluding smoking history as a factor, the OR of the females and patients with adenocarcinoma was 3.81 (95% CI: 1.36–10.7, $P = 0.011$) and 6.45 (95% CI: 0.76–55.6, $P = 0.087$), respectively.

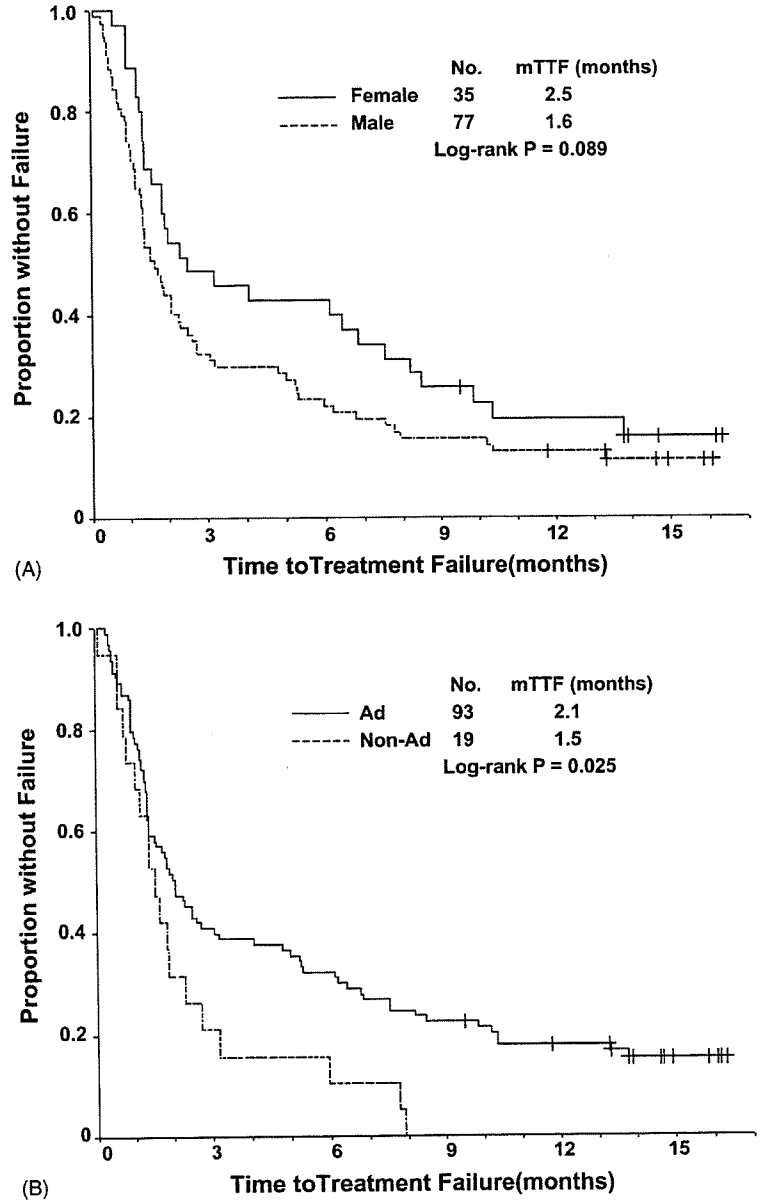

Fig. 1 Kaplan–Meier plot of time to treatment failure according to subgroups: (A) female versus male; (B) adenocarcinoma versus non-adenocarcinoma; (C) never-smokers versus moderate/heavy smokers. mTTF: median time to treatment failure, Ad: adenocarcinoma.

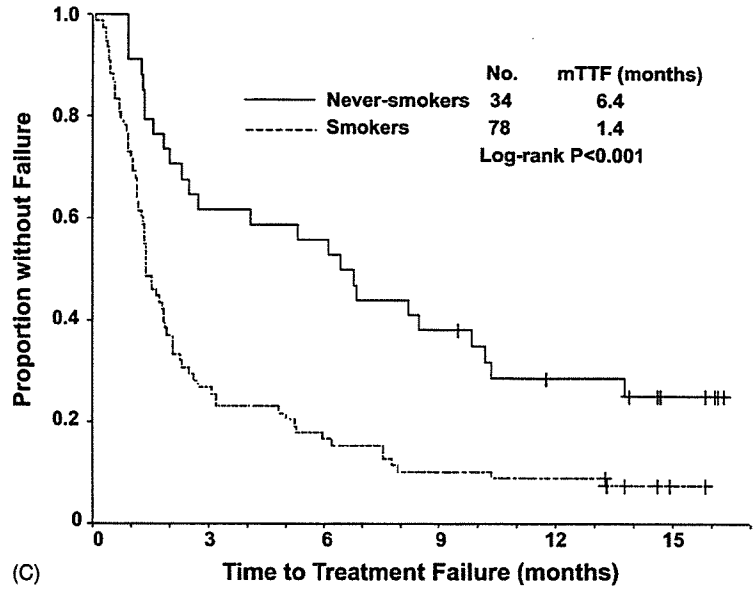

Fig. 1 (*Continued*).

The median follow-up time for survivors was 14.7 months, and ranged from 11.0 to 16.8 months. Sixty-nine patients (62%) died: 65 of disease progression and 4 of toxicity. Gefitinib treatment was terminated in 97 patients (87%) because of disease progression (68 patients), no tumor shrinkage (7 patients), toxicity (19 patients), or at the patients' request (3 patients). The median TTF and the median survival time (MST) for all patients were 1.9 and 10.7 months, respectively. The 1-year survival rate was 45%. The Kaplan–Meier plots of TTF and OS in each subgroup are shown in Figs. 1 and 2. The women had a longer TTF and OS than the men, but the difference was not significant. Patients with adenocarcinoma had a significantly longer TTF than those with non-adenocarcinoma, and "adenocarcinoma" was a marginally significant predictor of longer survival. "No history of smoking" was a highly significant predictor of longer TTF ($P < 0.001$) and longer survival ($P = 0.007$); the MST was 15.3 months in never-smokers and 8.8 months in moderate/heavy smokers.

We observed an association between efficacy and toxicity. As shown in Table 6, those who experienced skin rash or elevation in GOT/GPT levels tended to

Table 6 Association between efficacy and toxicity

	No. of patients	Response rate (%)	*P*-values*	Median survival (months)	1-year survival (%)	*P*-values†
Skin rash						
Grade 0	21	12	0.043	3.0	24	0.011
Grade 1	59	33		10.6	44	
Grade 2	29	46		15.3	66	
Diarrhea						
Grade 0	48	33	0.903	9.3	35	0.037
Grade 1–2	61	32		13.6	54	
GOT/GPT						
Grade 0	57	21	0.004	7.8	31	0.006
Grade 1	31	48		15.1	55	
Grade 2–3	18	50		Not reached	83	

* *P*-values for chi-square test between grade 0 and 1–3.
† *P*-values for log-rank test.