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III. 研究成果の刊行物・別刷

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Pleural Lavage Cytology Before and After Lung Resection in Non-Small Cell Lung Cancer Patients

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Background. The aim of this study was to analyze on a multivariate basis the prognostic significance of pre-resection and post-resection pleural lavage cytologies in surgically resected primary non-small cell lung cancer (NSCLC) patients, in relation to pathologic TNM factors in a large cohort of almost 1,200 patients.

Methods. From August 1992 through March 2001, pleural lavage cytology (PLC) was performed in 1,214 NSCLC patients without pleural effusion or dissemination undergoing pulmonary resection. The cytologic evaluation was classified into three categories: negative, suggestive, and positive. To investigate the impact on patient survival, PLC results were analyzed with conventional clinicopathologic factors.

Results. Definitive pre-resection PLC result was obtained in 1,194 patients and 38 had a positive result. The 5-year survival rates were 27% if pre-resection PLC was positive and 71% if negative. Of 1,198 patients 54 had a positive post-resection PLC result. The 5-year survival rates were 10% if post-resection PLC was positive and 73% if negative. On multivariate analysis, post-resection PLC was an independent prognostic factor as significant as established clinicopathologic factors.

Conclusions. Pre-resection and post-resection PLC should be recognized as an essential prognostic factor and should be performed in NSCLC patients without pleural effusion and dissemination. Post-PLC, compared with pre-PLC, had a greater and independent impact on survival and needs to be incorporated in the pathologic staging of NSCLC in the future.

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Pleural lavage cytology (PLC) has been reported to be a possible prognostic factor in patients with resected non-small cell lung cancer (NSCLC). However, many of the reports are that only PLC immediately after thoracotomy, before lung resection, (pre-PLC) has been studied in detail. The pre-PLC impact on patient outcome has been studied, chiefly, on a univariate basis and has not been studied in relation to the conventional pathologic TNM by multivariate analysis. Although pre-PLC has been reported to be a poor prognosis predictor, a positive result is currently not recognized as equivalent to T4 or a factor indicating incomplete resection. Although PLC after radical NSCLC resection, before chest closure, (post-PLC) has also been studied, significance of post-PLC remains controversial. Higashiyama and associates [1] performed pre-PLC and post-PLC in 325 lung cancer patients, but neither pre-PLC nor post-PLC results were an independent prognostic factor. Dresler and associates [2], who reported the pre-PLC and post-PLC analysis in 137 patients, stated that the 3-year survival rate was significantly better in negative post-PLC patients than in positive patients. We thought further analyses on post-PLC were needed. In the present study, we analyzed both pre-PLC and post-PLC on a multivariate basis, in relation to pathologic TNM factors in a large cohort of almost 1,200 patients.

Material and Methods

From August 1992 through March 2001, a total of 1,387 patients underwent surgical resection for primary NSCLC at the National Cancer Center Hospital East. Intraoperative PLC, which was approved for this observational study by the institutional review board, was prospectively performed in all patients without pleural effusion and dissemination, totaling 1,214 patients, and all were enrolled in this study. As the largest sample size for PLC study was 1,000 before this study, we aimed at accruing well more than 1,000 patients before analysis. Preoperative evaluation included a detailed history, physical examination, bronchoscopy, contrast-enhanced computed tomography (CT) of the chest, and distant metastasis screening (bone, brain, liver, and adrenals). Histologic typing was determined according to the World Health Organization classification [3]. Disease stages were determined based on the TNM classification of the International Union Against Cancer [4]. Immediately after

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thoracotomy, the pleural cavity was carefully washed with 500 mL physiologic saline before any pulmonary parenchyma manipulation. A sample of 50 mL was retrieved for cytologic evaluation (pre-PLC). We performed lung resection (segmentectomy or greater) and complete mediastinal lymph node dissection in 1,199 patients, and lung resection and mediastinal lymph node sampling in 15 patients. Before chest closure, a pleural cavity lavage sample was also retrieved (post-PLC) in the same fashion as pre-PLC. Samples were centrifuged at 1,500 rpm for 5 minutes. The sediment was stained using Papanicolaou's methods. A single cytologist blinded to the clinical-pathologic information evaluated the specimen and classified it into three categories: Papanicolaou classes I and II as negative, class III as suggestive, and classes IV and V as positive. In the survival analyses, we studied only cases with definitive cytologic diagnoses, excluding Papanicolaou class III. To investigate the impact on patient survival, the following conventional clinicopathologic factors were reviewed and analyzed: age, gender, smoking index (< 400 vs ≥ 400), serum carcinoembryonic antigen (CEA) level (< 5.0 mg/mL vs ≥ 5.0 mg/mL), clinical T factor (cT: cT2-4 vs cT1), clinical lymph node status (cN: mediastinal node involvement as cN2 vs less extensive as cN0-1), histologic type of tumor (adenocarcinoma versus others), pleural involvement of surgical (sP0-1 vs sP2-3) and pathologic finding (p0 vs p1-3), lymphatic invasion (positive versus negative), vascular invasion (positive versus negative), pathologic N status (pN: pN2-3 vs pN0-1), degree of fibrotic scarring (scar grade 1-2 vs grade 3-4), nuclear atypia (grade 1 or 2 vs grade 3), mitotic activity (mitotic index 1 or 2 vs 3), and surgical resection completeness (incomplete versus complete). Complete resection was defined as negative surgical margin and no highest mediastinal lymph node involvement. Incomplete resection was defined as positive surgical margin or highest mediastinal lymph node involvement. The smoking index was defined as the product of the number of cigarettes smoked per day and the number of years of smoking. We defined cN2 as mediastinal lymph node(s) greater than 1.0 cm in the shortest dimension on preoperative conventional CT. Pleural involvement was classified according to the Japan Lung Cancer Society criteria: p0; tumor did not extend beyond the elastic pleural layer, p1; tumor invaded the visceral pleura elastic layer but was not exposed on the pleural surface, p2; tumor was exposed on the pleural surface and p3; tumor invaded the parietal pleura or chest wall. Surgeons determined pleural involvement (sP factor) macroscopically before resection. Pathologic pleural involvement (p factor) were diagnosed on the resected specimens by a single pathologist blinded to the surgeons' findings [5]. Lymphatic invasion and vascular invasion indicated tumor cells identifiable in the lymphatic and vascular vessel lumen, respectively. Scar grade was classified into 4 grades: grade 1; tumor had foci of alveolar collapse with resulting condensation of elastic fibers but no or minimal fibroblastic tissue with collagen, grade 2; tumor had fibroblastic tissue with a small amount of collagen fibers, grade 3; tumor had fibroblastic tissue with moderate or abundant amount of collagen fibers, and grade 4; tumor showed hyalinization [6]. Nuclear atypia categorization was based on the most atypical nuclei on sections and divided into 3 grades as follows: grade1; nuclei that were uniform in size and equal to or only slightly larger than those of reactive type II alveolar epithelial

Table 1. Patient Characteristics ($n = 1,214$)

Characteristics	Results	
Gender		
Male	781	(64)
Female	433	(36)
Histology		
Adenocarcinoma	792	(65)
Squamous cell carcinoma	284	(23)
Others	138	(12)
Clinical T factor		
T1	593	(49)
T2	490	(40)
T3	111	(9)
T4	20	(2)
Clinical N factor		
N0	1,005	(83)
N1	116	(10)
N2	92	(8)
N3	1	(<1)
Clinical stage		
IA	550	(45)
IB	376	(31)
IIA	17	(1)
IIB	129	(11)
IIIA	113	(9)
IIIB	24	(2)
IV	5	(<1)
Pathologic T factor		
T1	543	(45)
T2	434	(36)
T3	126	(10)
T4	111	(9)
Pathologic N factor		
N0	801	(66)
N1	204	(17)
N2	202	(17)
N3	7	(1)
Pathologic stage		
IA	438	(36)
IB	256	(21)
IIA	51	(4)
IIB	147	(12)
IIIA	196	(16)
IIIB	113	(9)
IV	13	(1)

(Numbers in parentheses are percentages)

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Table 2. Pre-PLC Result and Clinicopathologic Characteristics

Factors	Pre-PLC (n = 1,194)		*P* Value
	Positive (n = 38)	Negative (n = 1,156)	
Age	63	63	0.740
Gender			
Male	25	746	0.873
Female	13	410	
Treatment modality (resection type)			
Lobectomy	34	1,049	0.177 (limited resection vs others)
Pneumonectomy	1	64	
Limited resection	3	43	
Pathologic stage			
I	16	667	0.056 (stage I vs others)
II	3	193	
III	19	283	
IV	0	13	
Histology			
Adenocarcinoma	26	751	0.660 (adenocarcinoma vs others)
Squamous cell carcinoma	6	274	
Large cell carcinoma	3	47	
Other	3	84	
Pathologic pleural involvement			
p0	11	754	<0.001
p1–3	27	402	
Pathologic N status			
N0	17	774	0.041
N1–3	21	382	
Lymphatic invasion			
Positive	27	481	<0.001
Negative	11	675	
Vascular invasion			
Positive	30	633	0.003
Negative	8	523	
Resection completeness			
Complete	28	1,067	<0.001
Incomplete	10	89	
Scar grade			
1–2	0	191	0.001
3–4	35	844	
NA	3	121	
Nuclear atypia			
1–2	15	432	0.863
3	20	607	
NA	3	117	
Mitotic index			
1–2	26	813	0.539
3	9	226	
NA	3	117	

NA = data not available.

cells, grade 2; nuclei that were uniform in size and up to twice the size of those of reactive type II alveolar epithelial cells, and grade 3; presence of giant tumor cells. Mitotic index was classified into three grades based on the findings of several sections: index 1; up to 5 mitotic cells per 10 high-power fields (HPF), index 2; 6–15 mitotic cells per 10 HPF, and index 3; greater than 15 mitotic cells per 10 HPF [7]. The length of survival was defined as the interval in months between the day of surgical intervention and the date of death due to

Table 3. Post-PLC Results and Clinicopathologic Characteristics

Factors	Post-PLC (n = 1,182)		
	Positive (n = 54)	Negative (n = 1,128)	*p* Value
Age	61	63	0.363
Gender			
Male	37	725	
Female	17	403	0.524
Treatment modality (resection type)			
Lobectomy	48	1,026	
Pneumonectomy	2	63	0.129
Limited resection	4	39	(limited resection vs others)
Pathologic stage			
I	7	673	
II	3	191	<0.001
III	42	253	(stage I vs others)
IV	2	11	
Histology			
Adenocarcinoma	41	731	
Squamous cell carcinoma	4	270	0.094
Large cell carcinoma	4	46	(adenocarcinoma vs others)
Other	5	81	
Pathologic pleural involvement			
p0	26	732	
p1–3	28	396	0.019
Pathologic N status			
N0	10	776	
N1–3	44	352	<0.001
Lymphatic invasion			
Positive	43	463	
Negative	11	665	<0.001
Vascular invasion			
Positive	41	614	
Negative	13	514	0.002
Resection completeness			
Complete	25	1,001	
Incomplete	29	127	<0.001
Scar grade			
1–2	3	186	
3–4	47	821	0.022
NA	4	121	
Nuclear atypia			
1–2	18	423	
3	32	588	0.465
NA	4	117	
Mitotic index			
1–2	42	790	
3	8	221	0.382
NA	4	117	

NA = data not available.

any cause or the last follow-up. An observation was censored at the last follow-up when the patient was alive or lost to follow-up. The survival rates were calculated by the Kaplan-Meier method [8] and univariate analyses were performed by means of the log-rank test. Multivariate analyses were performed using the Cox proportional hazards model [9]. Forward and backward stepwise procedures were used to determine the combination of prognostic factors (StatView: version 5.0; SAS Institute, Inc, Cary, NC). A *p*

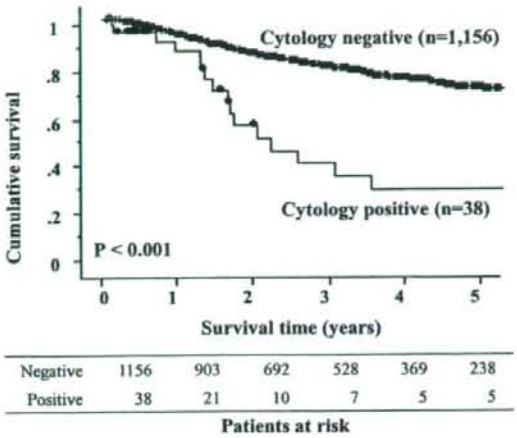

Fig 1. Survival curves of patients according to pre-PLC results. The 5-year survival rate was 27% for positive pre-PLC patients and was significantly worse (71%) for negative pre-PLC patients. The crosses indicate censored cases at the respective points. (PLC = pleural lavage cytology.)

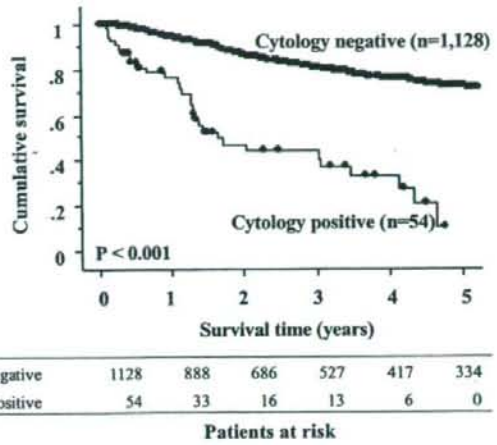

Fig 2. Survival curves of patients according to post-PLC results. The 5-year survival rate was 10% for positive post-PLC patients and was significantly worse (73%) for negative post-PLC patients. The crosses indicate censored cases at the respective points. (PLC = pleural lavage cytology.)

value less than 0.05 was taken to indicate a statistical significance.

Results

Patient clinicopathologic characteristics are shown in Table 1. There were 781 men and 433 women. Their ages ranged from 22 to 89, with a median of 65 years. Clinicopathologic characteristics for pre-PLC and post-PLC are shown in Tables 2 and 3, respectively. For pre-PLC, definitive cytologic results were obtained in 1,194 patients, with a positive result in 38 (3.2%). Univariate analyses revealed significant differences between pre-PLC positive and negative patients in pathologic pleural involvement, pathologic N status, lymphatic permeation, vascular invasion, resection completeness, and scar grade. For post-PLC, definitive cytologic result was obtained in 1,182 patients, 54 (4.6%) of which showed a positive result. Significant differences were observed in pathologic stage, pathologic pleural involvement, pathologic N status, lymphatic permeation, vascular invasion, resection completeness, and scar grade between post-PLC positive and negative patients. The 5-year survival rate was 27% for positive pre-PLC patients, which was significantly worse than 71% for negative pre-PLC patients (Fig 1). The 10% 5-year survival rate for positive post-PLC patients was significantly worse 73% for negative post-PLC patients (Fig. 2).

Five-year survival rates for patients with negative pre-PLC and post-PLC (n = 1,094), positive pre-PLC and negative post-PLC (n = 21), negative pre-PLC and positive post-PLC (n = 37), and positive pre-PLC and positive post-PLC (n = 13) were 81, 50, 12, and 0%, respectively. Multivariate analyses revealed 6 independent prognostic factors when only factors available before lung resection were analyzed (Table 4): age, CEA level, cT factor, cN factor, sP factor, and pre-PLC result. When factors available after postoperative pathologic evaluation were included in multivariate analyses, however, 10 independent prognostic factors were recognized, but pre-PLC result was not (Table 5): Age, CEA level, cT factor, pT factor, pN factor, p factor, lymphatic invasion, vascular invasion, resection completeness, and post-PLC result.

Comment

The first report on PLC was in 1958 by Spjut and associates [10]. They reported the results of post-PLC in 49 patients with lung cancer undergoing surgical resection. The cytologic results were positive for malignant cells in 16 (33%) of them, but outcomes were not analyzed. In 1984, Eagan and colleagues [11] reported positive post-PLC in 12 (8.9%) of 135 patients. Lung cancer recurred in nine of the 12 patients, with only two in the

Table 4. Multivariate Analysis Results for Prognostic Factors Available Before Lung Resection

Variable	Hazard Ratio (95% CI)	*p* Value
Age	1.020 (1.006–1.035)	0.005
Gender	0.958 (0.638–1.436)	0.833
Smoking (S.I > 400)	0.963 (0.648–1.433)	0.853
CEA	1.732 (1.320–2.272)	<0.001
cT factor (2–4 vs 1)	0.624 (0.475–0.814)	0.002
cN factor (1–3 vs 0)	0.512 (0.379–0.691)	<0.001
sP factor (2–3 vs 1–2)	0.621 (0.475–0.814)	<0.001
Pre-PLC	2.980 (1.683–5.277)	<0.001

CEA = serum carcinoembryonic antigen; CI = confidence interval; PLC = pleural lavage cytology; S.I = smoking index.

Table 5. Multivariate Analysis Results Including Factors Available After Lung Resection

Variable	Hazard Ratio (95% CI)	*p* Value
Age	1.021 (1.006–1.037)	0.006
CEA	1.301 (0.970–1.744)	0.079
cT factor (2–4 vs 1)	0.971 (1.411–2.051)	0.071
cN factor (1–3 vs 0)	0.951 (0.652–1.388)	0.796
sP factor (1–3 vs 0)	1.244 (0.834–1.856)	0.284
pT factor (2–4 vs 1)	1.285 (1.181–1.399)	<0.001
pN factor (1–3 vs 0)	0.446 (0.316–0.629)	<0.001
p factor (1–3 vs 0)	0.726 (0.527–1.001)	0.050
Histology (Ad.* vs others)	1.100 (0.769–1.573)	0.602
Lymphatic invasion	1.495 (1.058–2.114)	0.023
Vascular invasion	2.161 (1.410–3.311)	<0.001
Scar grade (3–4 vs 1–2)	0.792 (0.453–1.383)	0.412
Nuclear atypia (3 vs 1–2)	0.634 (0.447–0.898)	0.010
Mitotic index (3 vs 1–2)	0.875 (0.617–1.239)	0.452
Resection completeness	0.676 (0.472–0.968)	0.033
Pre-PLC	1.833 (0.949–3.541)	0.071
Post-PLC	1.803 (1.077–3.018)	0.024

Ad.* = adenocarcinoma; CEA = serum carcinoembryonic antigen; PLC = pleural lavage cytology.

ipsilateral pleural space. Eight patients died of lung cancer, one recurring locally and seven having distant metastases. They concluded the prognostic role of PLC needed further study. The first report on pre-PLC was by Kondo and associates in 1989 [12], followed by their expanded result analyses in 1993 [13]. They reported that 42 (9.0%) of 467 lung cancer patients undergoing surgery with little or no pleural effusion had a positive pre-PLC result. The 3-year survival rates of the patients with negative and positive cytology results were 68.7% and 22.9%, respectively. The prognosis of the positive cytology group was as poor as that of stage IIIB or IV patients. They concluded that pre-PLC was an important prognostic factor, indicating microscopic cancer cell exfoliation into the pleural cavity and subclinical malignant pleural effusion. Okada and associates [14] reported, based on 1,000 patients in 2003, that 45 (4.5%) patients had positive pre-PLC findings. Positive cytologic findings were observed more frequently in patients with adenocarcinoma, advanced stage, extended lymph node involvement, pleural involvement, lymphatic invasion, vascular invasion, high serum CEA level, and male gender. The survival rate at 5 years was 28% in patients with a positive result and 67% in negative patients ($p < 0.001$). Multivariate analysis demonstrated that pre-PLC was an independent prognostic determinant ($p = 0.0290$). Higashiyama and associates [1] performed pre-PLC and post-PLC in 325 lung cancer patients without malignant pleurisy. Positive post-PLC patients especially with adenocarcinoma resulted in a poor outcome. The survival rate at 5 years was 71% in 250 patients with negative pre-PLC and post-PLC results, while it was 33% in 19 patients with positive results. However, in multivariate analyses, neither pre-PLC nor post-PLC result was an independent prognostic factor in their study. Dresler and associates [3] reported the pre-PLC and post-PLC analysis in 137 patients in 1999. The 3-year survival rates of the patients with negative and positive pre-PLC results were 55% and 0%, respectively ($p = 0.088$). The 3-year survival rates of the patients with negative and positive post-PLC results were 50% and 0%, respectively ($p < 0.04$). In the present study, we analyzed both pre-PLC and post-PLC in almost 1,200 patients, the largest cohort ever studied with regard to PLC. Both pre-PLC and post PLC were analyzed in a multivariable setting, together with conventional significant clinicopathologic prognostic factors we reported previously [15]. Although our study yielded results similar to previous studies and post-PLC proved to be an important prognostic predictor, we found no difference in PLC results in relation to histologic characteristics. There have been a considerable number of reports concluding positive pre-PLC to be a poor prognosis predictor since pre-PLC was first reported by Kondo and associates in 1989 [12]. However, positive pre-PLC is currently not recognized as equivalent to T4 or a factor indicating incomplete resection [16–18]. In our study, pre-PLC was an independent prognostic factor when analyzed with prognostic factors available before lung resection, but not when postoperative pathologic factors and post-PLC results were combined in analyses. Positive pre-PLC patient outcome, when post-PLC was negative, was not very poor, with the 5-year survival rate reaching almost 60%. Therefore, positive pre-PLC result alone does not contraindicate surgical resection. In contrast, post-PLC proved to be an independent prognostic factor as significant as other established prognostic factors, including pathologic TNM status. No positive post-PLC patients survived beyond 4 years. As the patient outcome was extremely poor when pre-PLC was also positive, adjuvant therapy may be needed in these patients. We conclude PLC should be recognized as an essential prognostic factor and should be performed in NSCLC patients without pleural effusion and dissemination. And post-PLC, compared with pre-PLC, had a greater and independent impact on survival and needs to be incorporated in the pathologic staging of NSCLC in the future. As Vicidomini and associates referred to in their recent article on PLC [19], the results of the American College of Surgeons Oncology Group's Z0040 trial, which has completed a 1,200 patient accrual, will further define the potential implications of PLC in the management of lung cancer.

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ORIGINAL REPORT

First-Line Single Agent Treatment With Gefitinib in Patients With Advanced Non–Small-Cell Lung Cancer: A Phase II Study

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ABSTRACT

Purpose
We conducted a phase II study of single agent treatment with gefitinib in chemotherapy-naïve patients with advanced non–small-cell lung cancer (NSCLC) to assess its efficacy and toxicity.

Patients and Methods
Patients received 250 mg doses of gefitinib daily. Administration of gefitinib was terminated if partial response (PR) was not achieved within 8 weeks or if tumor reduction was not observed within 4 weeks. In these cases, platinum-based doublet chemotherapy was given as a salvage treatment. We evaluated mutation status of the epidermal growth factor receptor (EGFR) gene in cases with available tumor samples.

Results
Forty-two patients were enrolled between March and November 2003, with 40 of these patients being eligible. The response rate was 30% (95% CI, 17% to 47%). The most common toxicity included grade 1 or 2 acne-like rash (50%) and grade 1 diarrhea (18%). Grade 2 or 3 hepatic toxicity was observed in 8% of patients. Four patients developed grade 5 interstitial lung disease (ILD). Thirty patients received second-line chemotherapy. Median survival time was 13.9 months (95% CI, 9.1 to 18.7 months), and the 1-year survival rate was 55%. Tumor samples were available in 13 patients, including four cases of PR, six cases of stable disease, and three cases of progressive disease. *EGFR* mutations (deletions in exon 19 or point mutations [L858R or E746V]) were detected in four tumor tissues. All four patients with *EGFR* mutation achieved PR with gefitinib treatment.

Conclusion
Single agent treatment with gefitinib is active in chemotherapy-naïve patients with advanced NSCLC, but produces unacceptably frequent ILD in the Japanese population.

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INTRODUCTION

Previous meta-analysis demonstrated that cisplatin-based chemotherapy yielded a modest but significant survival benefit over best supportive care in advanced non–small-cell lung cancer (NSCLC).[1-4] In the 1990s, new agents, including vinorelbine, gemcitabine, paclitaxel, docetaxel, and irinotecan became available for the treatment of NSCLC. Several phase III trials comparing doublet platinum-based chemotherapies demonstrated no significant difference with respect to response rate, survival, or quality of life.[5,6] Nonplatinum or triplet platinum-based combination chemotherapies have been investigated, but none of these produced longer survival than standard doublet platinum-based chemotherapy.[7-9]

Recently, molecular-targeted agents have been introduced for the treatment of NSCLC. Gefitinib is an orally active epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor, which displays activity against recurrent NSCLC after platinum-based chemotherapy. Two international, randomized phase II trials in patients with advanced or metastatic NSCLC after platinum-based chemotherapy demonstrated response rates of 12% to 18% (28% in the Japanese population).[10,11] Two international, randomized, double-blinded, placebo-controlled phase III trials investigated the role of gefitinib combined with platinum-based chemotherapy regimens, including carboplatin and paclitaxel, or cisplatin and gemcitabine in chemotherapy-naïve patients with advanced NSCLC.[12,13] Surprisingly, there were no improvements in overall survival,

time to progression, or response rate. There are no data available regarding first-line treatment with single agent gefitinib against NSCLC in the Japanese population. Here, we conducted a phase II study of single agent treatment with gefitinib in chemotherapy-naïve patients with advanced NSCLC. If a failure with gefitinib treatment was perceived, standard platinum-based doublet chemotherapy was performed as salvage. The primary end point of this phase II trial was response rate, and the secondary end points were toxicity, survival, and response rate of salvage chemotherapy.

PATIENTS AND METHODS

Patient Population

Patients were required to have histologically or cytologically confirmed stage IIIB (malignant pleural or pericardial effusion and/or metastasis in the same lobe) or stage IV NSCLC. Recurrences after surgical resection were permitted. Other criteria included: (1) age 20 years or older, but younger than 75 years; (2) Eastern Cooperative Oncology Group performance status (PS) 0 or 1; (3) measurable disease; (4) PaO2 $\geq$ 60 mmHg; (5) adequate organ function (ie, total bilirubin $\leq$ 2.0, AST and ALT $\leq$ 100 U/L, serum creatinine $\leq$ 1.5 mg/dL, leukocyte count 4,000 to 12,000/mm^3, neutrophil count $\geq$ 2,000/mm^3, hemoglobin $\geq$ 9.5 g/dL, and platelets $\geq$ 100,000/mm^3); (6) no prior chemotherapy or thoracic radiotherapy; (7) no interstitial pneumonia or pulmonary fibrosis, as determined by chest x-ray; (8) no paralytic ileus or vomiting, (9) no symptomatic brain metastases, (10) no active infection; (11) no active concomitant malignancy; (12) no pregnancy or breast-feeding; (13) no severe allergy to drugs. Patients with PaO2 less than 60 mmHg were excluded, because those patients might have pulmonary fibrosis, which is a risk factor of interstitial lung disease (ILD).[14] All patients were required to provide written informed consent and the institutional review board at the National Cancer Center approved the protocol.

Treatment Plan

Treatment was started within a week after enrollment in the study. Patients received 250 mg of gefitinib orally daily. In the event of grade 3 or more and/or unacceptable toxicities, gefitinib was postponed until these toxicities were improved to grade 2 or less. Dose reduction was not performed. If treatment was postponed four times or more, the treatment was terminated. Therapy was continued unless the patient experienced unacceptable toxicity or progressive disease, partial response (PR) was not achieved within 8 weeks, or the sum of the longest diameters of the target lesions decreased less than 10% within 4 weeks. If the gefitinib treatment failed according to these criteria, platinum-based doublet chemotherapy was performed as a salvage regimen.

Previous trials of gefitinib for pretreated patients with NSCLC reported that most responding patients showed rapid tumor regression within 4 or 8 weeks.[11] Furthermore, most responses by gefitinib were extreme shrinkage of the tumor. Minor response, as frequently seen by the treatment with cytotoxic agents, was seldom experienced. Stable disease with gefitinib corresponded to no tumor reduction or slight progression. If patients with stable disease continued the treatment with gefitinib until progressive disease became obvious, those patients might not be able to receive platinum-based salvage chemotherapy because of poor PS due to progressive disease. Platinum-based combination chemotherapy is the standard care for patients with advanced NSCLC and good PS. Platinum-based chemotherapy was thought to be essential for patients with no response from the first-line single agent treatment with gefitinib. Therefore, we implemented these early stopping criteria for treatment with gefitinib.

Study Evaluations

Pretreatment evaluations consisted of a complete medical history, determination of performance status, physical examination, hematologic and biochemical profiles, arterial blood gas examination, ECG, chest x-ray, bone scan, and computed tomography (CT) scan of the chest, ultrasound or CT scan of the abdomen, and magnetic resonance imaging or CT scan of the whole brain. Evaluations performed included a weekly chest x-ray for 4 weeks, and once every 2 weeks for biochemistry, complete blood cell, platelet, leukocyte differential counts, physical examination, determination of performance status, and toxicity assessment. Imaging studies were scheduled to assess objective response every month.

Response and Toxicity Criteria

Response evaluation criteria in solid tumors (RECIST) guidelines were used for evaluation of antitumor activity.[15] The target lesions were defined as $\geq$ 2 cm in the longest diameter on CT scans. A complete response (CR) was defined as the complete disappearance of all clinically detectable tumors for at least 4 weeks. A PR was defined as an at least 30% decrease in the sum of the longest diameters of the target lesions for more than 4 weeks with no new area of malignant disease. Progressive disease (PD) indicated at least a 20% increase in the sum of the longest diameter of the target lesions or a new malignant lesion. Stable disease was defined as insufficient shrinkage to qualify for PR and insufficient increase to qualify for PD. Toxicity was graded according to the National Cancer Institute Common Toxicity Criteria version 2.0.

Mutation Analysis of the EGFR Gene

Tumor specimens were obtained during diagnostic or surgical procedures. Biopsied or surgically resected specimens were fixed with formalin or 100% methanol, respectively. Tumor genomic DNA was prepared from paraffin-embedded sections using laser capture microdissection in biopsied specimens or macrodissection in surgically resected specimens at Mitsubishi Chemical Safety Institute LTD. Exons 18, 19, and 21 of the *EGFR* gene were amplified and sequenced as previously described.[16]

Statistical Analysis

In accordance with the minimax two-stage phase II study design by Simon,[17] the treatment program was designed to refuse response rates of 10% (P_0) and to provide a significance level of .05 with a statistical power of 80% in assessing the activity of the regimen as a 25% response rate (P_1). The upper limit for first-stage drug rejection was two responses in the 22 assessable patients; the upper limit of second-stage rejection was seven responses within the cohort of 40 assessable patients. Overall survival was defined as the interval between enrollment in this study and death or the final follow-up visit. Median overall survival was estimated by the Kaplan-Meier analysis method.[18] Fisher's exact test was used in a contingency table.

RESULTS

Patient Population

A total of 42 patients were enrolled in this study between March and November, 2003, with 40 of these patients being eligible. One patient was found ineligible due to anemia, the other because spinal magnetic resonance imaging could not confirm a positive bone scan. Patient characteristics are listed in Table 1. Sixty percent of patients were male; median age was 61 years. The most common histologic subtype was adenocarcinoma (75%). Most patients (93%) had stage IV disease or recurrence after surgical resection. Eighty percent of patients were current or former smokers.

Efficacy

One patient (3%) has been receiving gefitinib after 22 months. Four patients suspended gefitinib for 11, 14, 27, or 29 days, because of liver dysfunction (n = 3) and fever due to urinary tract infection (n = 1). Thirty-nine patients terminated gefitinib because of progressive disease (n = 20), no tumor reduction within 4 weeks (n = 12), not achieving PR within 8 weeks (n = 1), toxicities including pulmonary (n = 3), nausea and vomiting (n = 1), rash (n = 1), or hepatic dysfunction (n = 1).

There were 12 PRs in 40 eligible patients, and the objective response rate was 30% (95% CI, 17% to 47%; Table 2). All but one

Table 1. Patient Characteristics

Characteristic	No. of Patients
Patients enrolled	42
Patients eligible	40
Sex	
Male	24
Female	16
Age, years	
Median	61
Range	44-74
Performance status	
0	14
1	26
Stage	
IIIB	3
IV	34
Recurrence after surgery	3
Histologic type	
Adenocarcinoma	30
Squamous cell carcinoma	3
Large cell carcinoma	7
Smoking history	
Current	27
Former	5
Never	8

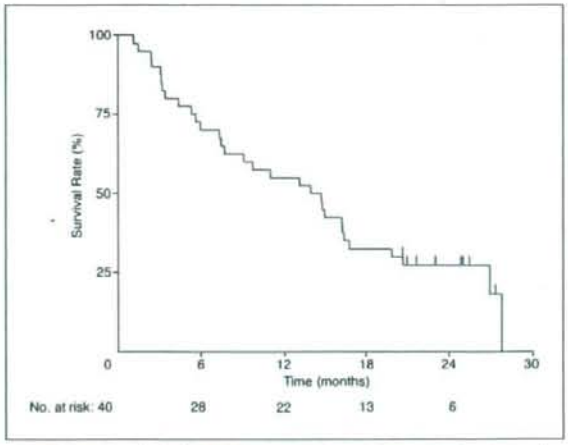

Fig 1. Overall survival of all eligible patients (n = 40) was calculated according to the Kaplan-Meier method. The median survival time was 13.9 months (95% CI, 9.1 to 18.7 months), and the 1-year survival rate was 55%.

patient from this subgroup achieved PR within 4 weeks, with the remaining patient achieving PR within 8 weeks. The background of the 12 responding patients was as follows: nine females, three males; 11 adenocarcinomas, one large-cell carcinoma; six individuals who never smoked, five current smokers, and one former smoker. Response rates based on patient characteristics were as follows: three of 24 (13%) males, nine of 16 (56%) females ($P = .0050$); 11 of 30 (37%) individuals with adenocarcinoma, one of 10 (10%) individuals with squamous or large-cell carcinoma ($P = .0048$); six of 32 (19%) current or former smokers, and six of eight (75%) individuals who never smoked ($P = .0048$).

The median follow-up time was 23 months, and nine patients were still alive at the most recent follow-up. The median survival time was 13.9 months (95% CI, 9.1 to 18.7 months), and the 1-year survival rate was 55% (Fig 1).

Safety and Toxicity

Toxicity was evaluated in all eligible patients. The most common toxicity was rash (Table 3). Thirty-eight percent and 13% of patients experienced grade 1 or 2 rash, respectively. One patient experienced grade 3 nausea and vomiting, leading to gefitinib treatment being terminated. Grade 3 hepatic toxicity was observed in one patient, also causing termination of gefitinib treatment.

The most problematic toxicity was ILD. We reviewed the medical records, chest x-rays, and CT films of all the cases, which were suspected as ILD by the physician in charge. ILD was diagnosed on the basis of standard or high-resolution CT findings of the chest (diffuse ground-glass opacity, consolidation, or infiltrate) and no response to antibiotics. We diagnosed that four patients experienced grade 5 ILD during or after first-line treatment with gefitinib. The first patient was a 61-year-old man. He developed dyspnea and fever elevation (38.1°C) on day 23 of the treatment with gefitinib and administration of gefitinib was terminated. Chest CT demonstrated bilateral diffuse ground-glass opacity, and PaO2 was 43.7mmHg in the room air. KL-6 antigen, a serum marker of interstitial pneumonia, was not elevated

Table 2. Efficacy of Single Agent Treatment With Gefitinib in Patients With Stage IIIb or IV Non-Small-Cell Lung Cancer

Type of Response	No. of Patients	% of Patients
Complete	0	0
Partial	12	30
CR + PR	12	30
95% CI	17 to 47	
Stable disease	16	40
Progression	12	30

Abbreviations: CR, complete response; PR, partial response.

Table 3. Maximum Toxicity Grades Associated With Single Agent Treatment With Gefitinib in 40 Patients With Non-Small-Cell Lung Cancer

	Toxicity Grade									
	1		2		3		4		5	
Toxicity	No. of Patients	%	No. of Patients	%	No. of Patients	%	No. of Patients	%	No. of Patients	%
Rash	15	38	5	13	0	0	0	0	0	0
Dry skin	4	10	0	0	0	0	0	0	0	0
Diarrhea	7	18	0	0	0	0	0	0	0	0
Nausea	3	8	0	0	1	3	0	0	0	0
Mucositis	6	15	0	0	0	0	0	0	0	0
Alopecia	4	10	0	0	0	0	0	0	0	0
Hyponatremia	24	60	0	0	3	8	0	0	0	0
Hypokalemia	12	30	0	0	0	0	0	0	0	0
Hepatic	11	28	2	5	1	3	0	0	0	0
Renal	4	10	1	3	0	0	0	0	0	0
ILD	0	0	0	0	0	0	0	0	4	10

Abbreviation: ILD; interstitial lung disease.

(351 U/mL) on day 24, but elevated on day 31 (1,400 U/mL). Beta-D-glucan, a serum marker of fungal infection and *Pneumocystis carinii* pneumonia, was also negative. Methylprednisolone and antibiotics were administered, with temporal improvement of ILD. However, subsequently, pulmonary function gradually deteriorated, leading to death. Autopsy revealed alveolar damage with organization around the bronchus and vessels in both neoplastic and non-neoplastic lesions, compatible with drug-induced ILD. The second patient was a 64-year-old man. Chest CT on day 27 showed stable disease, but administration of gefitinib was continued (protocol violation). Periodic chest x-ray film on day 45 showed abnormal shadow in the left lung field. High-resolution CT of the chest on the same day revealed reticular shadow on bilateral upper lobe. The treatment with gefitinib was terminated on day 45. KL-6 antigen was not elevated on day 49 (276 U/mL). Methylprednisolone and antibiotics were administered, but were not effective, leading to death. The third patient was a 67-year-old man. Chest CT on day 30 demonstrated enlargement of primary lesion and bilateral reticular shadow in subpleural lesions. Gefitinib was terminated on day 30. The patient developed dyspnea without fever elevation on day 37. Pao2 in the room air fell to 61.0 mmHg from 82.4 mmHg at pretreatment. Chest x-ray showed that the bilateral diffuse reticular shadow deteriorated. Methylprednisolone and antibiotics were administered, but were not effective, leading to death. Autopsy revealed severe fibrotic thickness of alveolar septum, compatible with severe interstitial pneumonia. There was no pathological evidence of carcinomatous lymphangiosis. The fourth patient was a 59-year-old woman. Chest x-ray showed consolidation in the left lung on day 21. Slight fever (37.9°C) developed on day 22. Blood culture was negative. Antibiotics were administered, but consolidation deteriorated and spread to both lungs on day 25. Gefitinib was terminated on day 25. KL-6 antigen was elevated to 3,590 U/mL. Methylprednisolone was administered, but was not effective, leading to death (Table 4). Four other patients experienced ILD after second-line or third-line chemotherapy. Two patients received second-line treatment with cisplatin plus vinorelbine (one and four courses), one patient received treatment with cisplatin plus gemcitabine (one course), and one patient received third-line treatment with docetaxel (four courses). Three of four patients received steroids, with temporal improvement of ILD being observed in two patients. However, ILD deteriorated during tapering of steroid treatment, with three patients subsequently dying. One patient stopped the third-line treatment with docetaxel, with the associated ILD showing improvement in this case without steroid treatment (Table 4).

We retrospectively reviewed the pretreatment chest x-rays and CT films of all patients. Interstitial shadow was not detected on pretreatment chest x-ray films in any patients. However, six patients showed evidence of interstitial shadow on pretreatment chest CT films. Three of the six patients with interstitial shadow, as determined by pretreatment chest CT, experienced ILD either during or following administration of gefitinib or second-line chemotherapy. None of the six patients responded to gefitinib treatment. On the other hand, four of 34 patients who showed no interstitial shadow on pretreatment chest CT films experienced ILD. Interstitial shadow as determined by pretreatment chest CT was not a statistically significant risk factor of ILD ($P = .0819$; Table 5).

Second-Line Chemotherapy

A total of 30 patients received second-line chemotherapy. Twenty-seven patients received platinum-based chemotherapy (cisplatin plus vinorelbine; n = 17), carboplatin plus paclitaxel (n = 5), cisplatin plus gemcitabine (n = 3), cisplatin plus docetaxel (n = 1), and cisplatin plus irinotecan (n = 1). The remaining three patients received vinorelbine plus gemcitabine or vinorelbine alone. Nine of 30 patients achieved PR with these second-line chemotherapies. The objective response rate of second-line chemotherapy was 30% (95% CI, 15% to 50%).

Mutation Status of the EGFR Gene

Out of 42 enrolled patients, 16 patients were diagnosed pathologically, 22 were diagnosed cytologically, and four patients recurred after surgical resection. Biopsied specimens were available in nine patients. Therefore, tissue samples were available in a total of 13 patients. These 13 patients included four PRs, six with stable disease, and three PDs. *EGFR* mutations were detected in four tumor tissues, including the in-frame nucleotide deletions in exon 19 (n = 3) and an L858R mutation in exon 21 (n = 1). One tumor had an in-frame deletion and

Table 4. Four Patients Developed Interstitial Lung Disease During First-Line Chemotherapy With Gefitinib, With Another Four Patients Showing ILD During Either Second- or Third-Line Chemotherapy

Age (years)	Sex	Smoking Index	Pathology	Onset of ILD	Response to Gefitinib	Death From Chemotherapy
61	M	1,520	AD	Day 23*	PD	Day 74
64	M	880	AD	Day 45*	SD	Day 51
67	M	1,880	SQ	Day 37†	PD	Day 45
59	F	0	AD	Day 21*	PD	Day 35
61	M	820	AD	Day 131‡	SD	Day 154
68	M	2,000	LA	Day 37†	PD	Day 106
68	M	705	AD	Day 225§	PR	Day 87
59	M	1,170	AD	Day 108‖	SD	Alive

Abbreviations: ILD, interstitial lung disease; M, male; F, female; AD, adenocarcinoma; SQ, squamous cell carcinoma; LA, large-cell carcinoma; PD, progressive disease; SD, stable disease; PR, partial response.
*During gefitinib administration.
†One week after discontinuation of gefitinib.
‡ After 2nd-line chemotherapy of cisplatin and vinorelbine.
§ After 2nd-line chemotherapy of cisplatin and gemcitabine.
‖ After 3rd-line chemotherapy of docetaxel.

Table 5. Interstitial Shadow on Pretreatment Chest Computed Tomography Films and ILD

Interstitial Shadow on Pretreatment Chest Computed Tomography Scans	No ILD	ILD
No existence	29	5
Existence	3	3

NOTE. $P = .0819$.
Abbreviation: ILD interstitial lung disease.

an E746V mutation in exon 19. All four PR patients had *EGFR* mutations (Table 6).

DISCUSSION

This phase II study was designed to evaluate the efficacy and safety of first-line single agent treatment with gefitinib in patients with advanced NSCLC. There is no other paper that evaluates single agent treatment with gefitinib prospectively in patients with advanced NSCLC. The observed response rate of 30% (95% CI, 17% to 47%), median survival of 13.9 months and 1-year survival of 55% are promising. However, grade 5 ILD occurred in 10% (95% CI, 3% to 24%) of patients. This high rate of ILD was not acceptable. The incidence of ILD was seen to be less than 1% in two randomized controlled studies comparing gefitinib with placebo in combination with gemcitabine and cisplatin or paclitaxel and carboplatin.[12,13] The reason for the high incidence of ILD observed in our study is unknown. The West Japan Thoracic Oncology Group analyzed 1,976 patients receiving gefitinib retrospectively. In this case, the incidence of ILD was 3.2% (95% CI, 2.5% to 4.6%) and the death rate due to ILD was 1.3% (95% CI, 0.8% to1.9%). Multivariate analyses found that risk factors included being male, individuals who smoked, and complication of interstitial pneumonia.[14] Our retrospective analyses revealed that three of six patients with interstitial shadow on pretreatment chest CT films, but not detected on chest x-ray films developed ILD; on the other hand, five of 34 patients without interstitial shadow developed ILD. Interstitial shadow on pretreatment chest CT was a marginally significant risk factor of ILD ($P = .0819$). It might be suggested that patients with interstitial shadow on pretreatment chest CT films be excluded from administration of gefitinib; however, our analyses were biased because we analyzed retrospectively and did not blind patient clinical information. Prospective analysis is needed to evaluate interstitial shadow by chest CT before treatment with gefitinib.

The Southwest Oncology Group conducted a phase II trial to evaluate gefitinib in patients with advanced bronchioloalveolar carcinoma (SWOG 0126). Previously untreated (n = 102) and treated (n = 36) patients were entered and eligible in SWOG 0126. The response rate was 19% and the median survival time was 12 months in the untreated population.[19] These subset analyses were comparable to our results.

Recently, mutations in the tyrosine kinase domain of EGFR were found to be associated with gefitinib sensitivity in patients with NSCLC.[16,20,21] Our retrospective analyses demonstrated that *EGFR* mutations were detected in four of 13 patients, and those four patients achieved PR in the single agent treatment of gefitinib. These results were compatible with previous reports.[16,20,21]

Thirty patients received second-line chemotherapy, including platinum-based (n = 27) and nonplatinum-based (n = 3) regimens; the response rate was 30%. Pretreatment with gefitinib does not seem to adversely affect the response of second-line chemotherapy. However, our small-scale study does not suggest the best second-line regimen. Platinum combined with any third-generation agents including paclitaxel, docetaxel, vinorelbine,

Table 6. Mutation Status of the *EGFR* Gene

Sex	Age (years)	Pathologic Type	Smoking Status	Overall Survival (months)	*EGFR* Gene	Effect of Mutation	Response to Gefitinib	Response to Second Line Chemotherapy
M	68	AD	Current	14.9	Deletion of 15 nucleotides (2236-2250)	In-frame deletion (E746-A750)	PR	PD
F	67	AD	Current	16.2	Deletion of 15 nucleotides (2236-2250)	In-frame deletion (E746-A750)	PR	PD
F	54	AD	Current	5.6	Deletion of 18 nucleotides (2238-2255) and substitution of T for A at nucleotides 2237	In-frame deletion (L747-S752) and amino acid substitution (P746V)	PR	NR
F	57	AD	Never	25.4	Substitution of G for T at nucleotide 2573	Amino acid substitution (L858R)	PR	SD
M	61	AD	Current	7.5	Wild	—	SD	SD
M	54	AD	Current	9.7	Wild	—	SD	SD
M	45	AD	Current	16.2	Wild	—	SD	PR
M	59	AD	Current	14.7	Wild	—	SD	PR
M	67	SQ	Current	2.4	Wild	—	SD	NR
M	59	AD	Current	24.9	Wild	—	SD	PR
M	61	AD	Current	2.4	Wild	—	PD	NR
F	61	SQ	Current	3.4	Wild	—	PD	PD
F	61	AD	Current	16.3	Wild	—	PD	PR

Abbreviations: EGFR, epidermal growth factor receptor; M, male; F, female; AD, adenocarcinoma; SQ, squamous cell carcinoma; PR, partial response; SD, stable disease; PD, progressive disease; NR, not received.

gemcitabine, or irinotecan is probably acceptable as the current standard first-line chemotherapy.

First-line single agent with gefitinib is active, but produces unacceptably frequent ILD in the Japanese population. Being female, as well as adenocarcinoma, those who never smoked, and *EGFR* mutation were associated with response to gefitinib. Patients who responded to gefitinib did not experience ILD during gefitinib chemotherapy. Further research via genetics and image analysis is needed to avoid ILD and identify a subgroup of patients that benefit from gefitinib treatment. If this is realized, single agent treatment with gefitinib could be an option as first-line chemotherapy in selected patients with advanced NSCLC. Furthermore, randomized trials are warranted to compare first-line single agent treatment with gefitinib followed by second-line platinum-based chemotherapy with first-line platinum-based chemotherapy followed by second- or third-line gefitinib treatment.

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Authors' Disclosures of Potential Conflicts of Interest

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