(ALK; 5.8%) (9, 10). Therefore, ethnic differences and driver mutations are important factors in NSCLC (particularly adenocarcinoma) treatment.

Recent reports revealed the impact of *EGFR* mutations, and *ALK* translocation on the efficacy of pemetrexed and P-C therapies (11-14). Moreover, ethnicity is a prognostic factor; East and South Asian patients had longer survival compared with that in the entire study population (Whites, African descent, East Asians, and others) in a phase III study (15) –and may be a predictive biomarker of enhanced sensitivity to pemetrexed. Although P-C provided tolerability in a phase I/ II study of malignant pleural mesothelioma in Japan (16), its overall efficacy, and that according to tumor genotype, has not been well-evaluated in Japanese patients with Nsq-NSCLC.

We conducted this prospective study to evaluate the efficacy and safety of P-C in Japanese patients with Nsq-NSCLC, and to determine whether the *EGFR* mutation or *ALK* translocation impacted the treatment outcome in these patients.

Patients and Methods

Eligibility. Patients with histologically- or cytologically-confirmed Nsq-NSCLC were eligible for this study. Additional eligibility criteria were as follows: clinical stage IIIB, IV, or recurrent disease, no prior chemotherapy, an Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1, and adequate bone marrow, renal, and hepatic function. Patients with unstable brain metastases, uncontrolled pleural effusion or ascites, active infection, active concomitant malignancy or interstitial pneumonia were excluded. The study protocol was approved by the Institutional Review Board at our center (UMIN000002847). All patients signed written informed consent before enrollment.

Treatment plan. Patients received pemetrexed (500 mg/m^2) intravenously (*i.v.*) for over 10 min followed by cisplatin (75 mg/m^2, *i.v.*) over 2 h on day 1 of a 21-day cycle. This combination therapy was repeated for up to four cycles. Patients were instructed to take oral multivitamin supplement (1 g/day) containing 500 mg folic acid beginning one week before the first treatment until 22 days after the last pemetrexed administration; vitamin B12 (1000 mg) was injected intramuscularly every nine weeks during the same period.

The second and subsequent treatment cycles were initiated only when the following criteria were satisfied on day 1 of the cycle: white blood cells ≥3,000/mm^3 or neutrophils ≥1,500/mm^3, platelets ≥100,000/mm^3, PS ≤1, creatinine ≤1.5 mg/dl, aspartate aminotransferase (AST)/alanine aminotransferase(ALT) ≤100IU/l, total bilirubin ≤1.5 mg/dl, body temperature(BT) <38°C, no interstitial pneumonia, non-hematological toxicity ≤G1. Pemetrexed was reduced to 400 mg/m^2 in the subsequent cycles if chemotherapy induced either grade 4 leukopenia or neutropenia for more than five days or grade 4 thrombocytopenia or thrombocytopenia requiring platelet transfusion, grade 3 febrile neutropenia or grade 3 non-hematological toxicities. Cisplatin was reduced to 60 mg/m^2 in the subsequent cycles if these toxicities recurred after the dose reduction of pemetrexed or if serum creatinine was more than 2.0 mg/dl. Patients would be withdrawn from the study if these toxicities recurred after the reduction in cisplatin dose, or if the next cycle was delayed because of toxicity for by more than 43 days

Evaluation of tumor response and toxicity. Complete patient histories, physical examinations, complete blood cell counts, serum electrolytes and chemistry were performed before initiation of treatment and before each treatment cycle. Tumor status and response were assessed by radiological examination, including computed tomography, at baseline and after every two treatment cycles. Response Evaluation Criteria in Solid Tumors (RECIST) criteria (version 1.0) (17) were used to define the antitumor effects, and toxicity was assessed based on the National Cancer Institute Common Toxicity Criteria (version 3.0) (18).

Detection of oncogene driver mutations. Genomic DNA was extracted from tumors embedded in paraffin blocks or from tumor cells from aspirates of pleural effusions, or biopsied superficial lymph nodes or subcutaneous metastases. Mutations in *EGFR* exons 19 and 21 were detected by the Cycleave real-time quantitative PCR technique, and the ALK translocation was examined using fluorescence *in situ* hybridization or highly sensitive immunohistochemistry (IHC) to detect the ALK fusion protein (19).

Statistical analysis. This study was a prospective, single-center, single-arm study (UMIN000002847) of first-line combination therapy withP-C. The primary end-point was the response rate (RR) and the secondary endpoints were toxicity, progression-free survival (PFS), and overall survival (OS).

A Simon's minimax two-stage phase II design (20) was used to define minimum sample sizes for statistical significance: assuming an expected overall RR of ≥50% and a minimum acceptable RR of 30%, 22 patients would be required as the first step. Our plan further stipulated that if at least seven out of the 22 patients responded to the therapy, another 24 patients would be required as the second step. If at least 17 of the 46 patients responded, the treatment would be declared sufficiently promising. OS was recorded as the time from registration until either death or conclusion of the analysis; PFS was the time from registration to documented progression or death from any cause, whichever occurred first. Survival analyses were performed using the Kaplan-Meier method. All statistical analyses were performed using the SPSS 17.0 statistical software (Dr SPSS II for Windows, Standard version 17.0; SPSS Inc., Chicago, IL, USA).

Results

Patients' characteristics. From November 2009 until January 2010, 50 patients with Nsq-NSCLC were enrolled. Patients' characteristics are listed in Table I. The median age was 60 years (range 28-74), and there were 34 males and 16 females. Thirty-one patients had PS 0 and 19 patients had PS 1. Forty-one patients (82.0%) had adenocarcinoma. *EGFR* mutation status was analyzed in 46 patients; nine patients (19.6%) harbored an activating mutation in *EGFR*. *ALK*-translocation was identified in six (15.4%) out of 39 patients analyzed. Six patients were positive by FISH, five out of six patients were positive by IHC, but the remaining patients were not evaluable by IHC.

Table I. *Patients' characteristics.*

Characteristic	N=50
Age, years	
Median (range)	60 (28-74)
Gender	
Male/female	34/16
Stage	
IIIB/IV/recurrence	5/40/5
History of smoking	
Non-smoker/smoker	14/36
Histology	
Adenocarcinoma/other	41/9
Performance status (ECOG)	
0/1	31/19
EGFR mutation status	
Positive/negative/unknown	9/37/4
ALK translocation status	
Positive/negative/unknown	6/33/11

ECOG: Eastern Cooperative Oncology Group; EGFR: epidermal growth factor receptor; ALK: anaplastic lymphoma kinase.

Table II. *Response rates.*

Response	N=50
CR	0
PR	22
SD	17
PD	10
NE	1
RR	44.0%
DCR	78.0%

NE, Not evaluable; DCR, disease control rate; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; RR, response rate.

Response and survival. Fifty patients were analyzed. There were no complete responses, 22 partial responses, 17 cases of stable disease, 10 of progressive disease, and one case was non-evaluable because of grade 5 pneumonitis during the first treatment cycle. Thus, the overall RR was 44.0% [95% confidence interval (CI)=30.0%-58.0%], and the disease control rate (DCR) was 78.0% (95% CI=66.0%-90.0%; Table II). At the median follow-up period of 19.0 months (range=1.4-35.2 months), the median PFS and OS were 4.3 months (95% CI=3.9-4.8 months; Figure 1A) and 22.2 months (95% CI=13.4-31.0 months; Figure 2B), respectively.

Treatment delivery. Thirty-three (66.0%) patients completed four cycles of P-C therapy. The median number of chemotherapy cycles administered throughout the study was four (range=1-4 cycles). However, one patient had dose reduction of cisplatin because of elevated serum creatinine level, two patients had dose reduction of pemetrexed because of infection (one patient) and fatigue (one patient).

Toxicity. Toxicity was evaluated in all patients in all cycles (Table III). Grade 3 or 4 neutropenia was observed in eight patients (16%) and grade 3 infection in three (6%), but there were no cases of febrile neutropenia. In addition, grade 3 or 4 anemia was observed in eight patients (16%), and grade 3 elevations of serum creatinine level were observed in two patients. Furthermore, one patient (2%) experienced grade 5 pneumonitis after one cycle.

Subgroup analysis by ALK fusion status and EGFR mutation status. Among the 39 out of 50 patients, we identified *ALK* translocations in six patients (15.4%), *EGFR* mutations in nine (23.1%), and wild-type *ALK* and *EGFR* in 24 patients (61.5%) (referred to as WT/WT). However, we were unable to examine *ALK* translocation in 11 patients and *EGFR* mutations in four patients because of insufficient material. Objective responses were observed in two patients with *ALK* translocation, six with *EGFR* mutation, and 11 (45.8%) of the WT/WT group. However, there were no significant differences in PFS by genotype. The median PFS in the *ALK* translocation, *EGFR* mutation and WT/WT subgroups were 3.0 months (95% CI=0.0-8.3 months), 5.5 months (95% CI=4.7-6.4 months) and 4.0 months (95% CI=2.9-5.1 months), respectively (Figure 1B). Median OS had not yet been reached in the patients with *EGFR* mutation and *ALK* translocation, and was 15.8 months in WT/WT patients (95% CI=2.9-28.8 months; Figure 2B).

Discussion

The impact of ethnicity and oncogene driver mutations on advanced NSCLC treatment has only recently begun to be considered. Although there have been several phase II and III studies of P-C for Nsq-NSCNC worldwide, there are no data for Japanese patients. In our phase II study of Japanese patients with Nsq-NSCLC, we observed that the efficacy of P-C in terms of overall RR and median PFS was comparable to those for other ethnicities, and we did not identify any new safety concerns. In our study, the overall RR was 44.0%, and the median PFS was 4.3 months, whereas in global studies, these values were 30.9%-45% and 5.3-6.3 months, respectively (4, 21, 22). Toxicities were mostly very mild; the major toxicities were myelosuppression, and the incidence of either grade 3 or grade 4 neutropenia or anemia were 16%. However, the toxicity profile was similar to that of previous studies: grade 3 or grade 4 neutropenia, 15.1%-58.3%; anemia, 5.6%-20% (4, 21, 22).

The median OS of our whole-patient sample was 22.2 months, which is significantly longer than that in previous

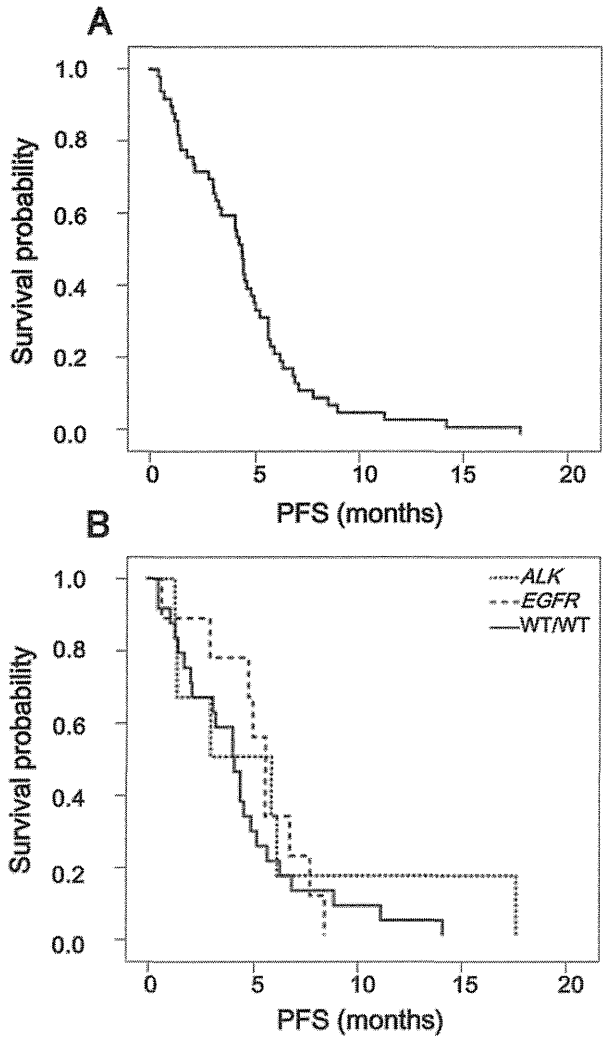

Figure 1. *Kaplan-Meier curves for progression-free survival (PFS) (N=50). The median PFS (N=50) was 4.3 months (A). The median PFS in subgroups of with patients with anaplastic lymphoma kinase (ALK) translocation, epidermal growth factor receptor (EGFR) mutation, wild-type for both ALK and EGFR (WT/WT) were 3.0, 5.5 and 4.0 months, respectively (B).*

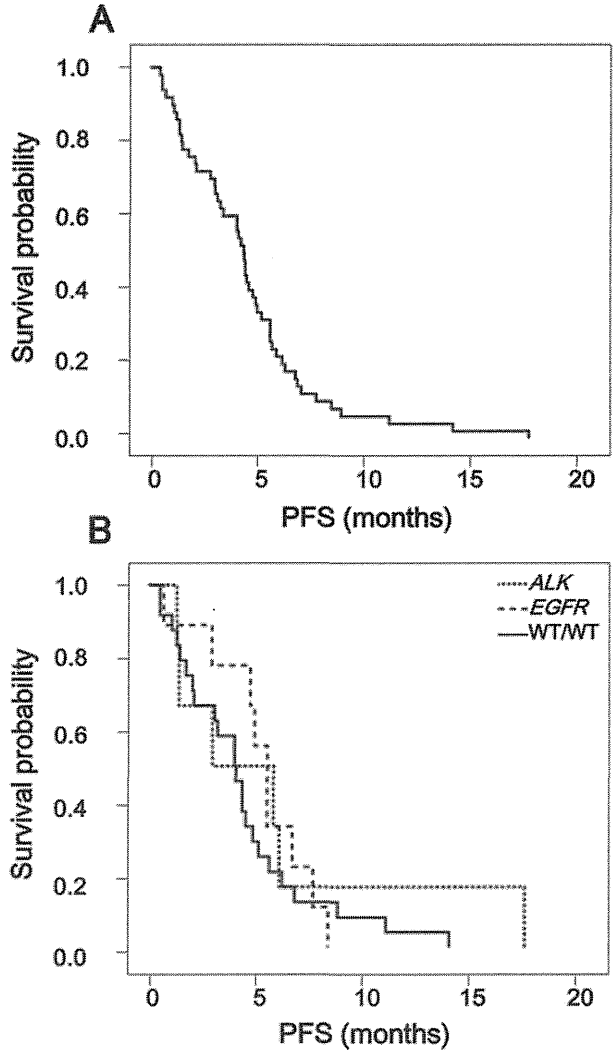

Figure 2. *Kaplan–Meier curves for overall survival (OS) (N=50). The overall median OS (N=50) was 22.2 months (A). The median OS had not yet been reached in the patients with epidermal growth factor receptor (EGFR) mutations or anaplastic lymphoma kinase (ALK) translocation, but was 15.8 months in wild-type for both ALK and EGFR (WT/WT) patients (B).*

reports (8.9-11.8 months) (4, 21, 22). Ethnic differences might have resulted in this discrepancy. Similar results were observed in a subset analysis of a previous global phase III study reporting that East Asian patients with Nsq-NSCLC (Taiwan and Korea) had longer median OS (21.2 months) than that of the population overall (23). In subset analysis of our study, patients with *EGFR* mutations or *ALK* translocations exhibited longer median OS compared with that in WT/WT patients. The driver mutation status and target therapy after the discontinuation of P-C may be related to prolonged survival. All patients with *ALK* translocations received ALK inhibitors for second-line or third-line treatment, and all patients with *EGFR* mutations received EGFR tyrosine kinase inhibitor for second-line treatment.

Some reports correlate *EGFR* mutations with the efficacy of pemetrexed. NSCLC cells with activating mutations in *EGFR* had lower TYMS expression than those with wild-type *EGFR* (24). In one study, patients with *EGFR* mutations receiving pemetrexed monotherapy responded more favorably and also had longer PFS than those with wild-type *EGFR* (14). TYMS is key folate enzyme targeted by pemetrexed and TYMS levels may correlate inversely with sensitivity to pemetrexed (25). In

Table III. *Hematological and non-hematological toxicities (N=50) experienced with pemetrexed-cisplatin therapy.*

	Grade*					
Toxicity	1	2	3	4	5	≥ Grade 3(%)
Hematological toxicity						
Leucopenia	18	11	1	0	0	2
Neutropenia	10	15	7	1	0	16
Anemia	21	16	3	5	0	16
Thrombocytopenia	9	3	0	2	0	4
Non-hematological toxicity						
Nausea	34	6	0	0	0	0
Vomiting	5	0	0	0	0	0
Diarrhea	5	1	1	0	0	0
Constipation	22	13	0	0	0	0
Rash	4	11	0	0	0	0
Elevated creatinine	12	1	2	0	0	0
Elevated aspartate aminotransferase	16	5	0	0	0	0
Elevated alanine aminotransferase	17	4	0	0	0	0
Infection	-	-	3	0	0	7
Pneumonitis	0	0	0	0	1	2

*National Cancer Institute-Common Terminology Criteria for Adverse Events (CTCAE) version 3.0 (18).

Table IV. *Patients' characteristics by genotype.*

Characteristic	All patients N=50	*ALK* translocation (n=6)	*EGFR* mutation (n=9)	WT/WT (n=24)
Age, years				
Median (range)	60 (28-74)	38 (28-67)	65 (37-72)	57 (41-73)
Gender				
Male/female	34/16	4/2	5/4	17/7
Stage				
IIIB/IV/recurrence	5/40/5	0/3/3	1/8/0	3/19/2
History of smoking				
Non-smoker/smoker	14/36	5/1	4/5	5/19
Histology				
Adenocarcinoma/other	41/9	6/0	9/0	18/6
Performance status (ECOG)				
0/1	31/19	6/0	8/1	14/10

ECOG: Eastern Cooperative Oncology Group, WT: wild-type.

our study, the RR was higher in the nine patients with EGFR mutations than in the entire study population (6/9 *vs.* 44.0%), although there were no differences in PFS.

ALK translocation has been identified as a driver mutation in NSCLC (26), and ALK tyrosine kinase inhibitors such as crizotinib have had a profound impact on the treatment of advanced NSCLC (27). Several retrospective studies report conflicting results on the efficacy of pemetrexed in *ALK*-positive patients. Camidge *et al.* reported that *ALK*-positive patients respond to pemetrexed with a better RR and longer PFS than WT patients (11). However, one of the largest retrospective analyses of pemetrexed-based chemotherapy documented no difference in PFS with respect to *ALK* status (13). In the phase III study of *ALK*-positive NSCLC comparing crizotinib, pemetrexed and docetaxel, overall RR and PFS were higher in the pemetrexed arm than in the docetaxel arm: 29.3% *vs.* 6.9% and 4.2 months *vs.* 2.6 months, respectively (28). Moreover, patients with *ALK* re-arrangements had lower TYMS expression than those with normal *ALK* loci. In our study, RR was lower in the six patients with *ALK* translocations than in the entire study population (6/9 *vs.* 44.0%), but there were no differences in PFS.

However, it is difficult to conclude on the mechanism of sensitivity to P-C therapy on the basis of the TYMS level in tumor tissue because of the lack of TYMS evaluation in our study. In addition, our study was too small to conclude whether particular genotypes correlate with the efficacy of P-C.

In conclusion, P-C therapy was effective and well-tolerated in Japanese patients with Nsq-NSCLC. We did not observe any obvious differences in the efficacy of P-C treatment between patients with *ALK* translocation or *EGFR* mutation status and these wild-type for these genes.

Disclosure

The Authors have declared no conflicts of interest.

Acknowledgements

The Authors thank all the patients and investigators who participated in this study.

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Received May 29, 2013
Revised June 27, 2013
Accepted June 28, 2013

Safety and Efficacy of Platinum Agents plus Etoposide for Patients with Small Cell Lung Cancer with Interstitial Lung Disease

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Abstract. *Background: The safety and efficacy of combination of platinum agents plus etoposide for patients with small cell lung cancer (SCLC) with pre-existing interstitial lung disease (ILD) is uncertain. Patients and Methods: Fifty-two patients received platinum agents plus etoposide as first-line chemotherapy for SCLC with pre-existing ILD. The clinical characteristics, treatment outcome and survival of these patients were retrospectively reviewed. Results: During first-line chemotherapy, only one (2%) out of the 52 patients developed an acute exacerbation of ILD. The median number of treatment cycles was four. The overall response rate was 69%. The median progression-free survival period was 4.5 months. The median survival time was 9.4 months. Thirty-three patients (63%) received at least one subsequent chemotherapy regimen, and five of these patients developed acute exacerbation of ILD. Conclusion: The combination of platinum agents plus etoposide is feasible and effective in SCLC patients with pre-existing ILD, compared with regimens after second-line chemotherapy.*

Small cell lung cancer (SCLC) accounts for 15% to 20% of all lung cancer cases (1). SCLC is characterized by rapid growth and widespread metastatic disease, and most patients have extensive disease at the time of diagnosis. SCLC is significantly sensitive to chemotherapy or radiation therapy, and therefore systemic chemotherapy is recognized as a standard treatment (2). The standard chemotherapy regimen for SCLC patients is the combination of platinum agents plus etoposide or platinum agents plus irinotecan, which is the most frequently used combination and yields a median survival period of approximately 9-12 months in clinical trials (3, 4).

Pre-existing interstitial lung disease (ILD) is one of the most common complications in patients with lung cancer. ILD, also known as diffuse parenchymal lung disease, is a diverse group of pulmonary disorders classified together because of similar clinical, radiographical, physiological, and pathological features (5). In patients with cancer, pre-existing ILD is considered to be a risk factor for acute exacerbation, which is a fatal complication of treatments such as chemotherapy, surgery, and radiation therapy (6, 7). The incidence of lung cancer in patients with ILD is reported to be 20-30% and is higher than that in the general population (8). Kudoh *et al.* reported recently that pre-existing ILD was confirmed to be an important determinant of the development of the acute exacerbation of ILD after chemotherapy for patients with advanced non-small cell lung cancer (NSCLC) (6). However, few reports exist on the association between pre-existing ILD and the safety and efficacy of chemotherapy in patients with SCLC. Whether chemotherapy for patients with SCLC with pre-existing ILD is feasible remains unclear because patients with severe complications, such as pre-existing ILD, have been excluded from most prospective clinical trials.

In this retrospective study, we investigated the safety and efficacy of the combination of platinum agents plus etoposide as a first-line chemotherapy for patients with SCLC with preexisting ILD.

Patients and Methods

Between January 2001 and December 2009, a total of 557 consecutive patients were diagnosed as having SCLC at the National Cancer Center Hospital East. Overall, 52 (11%) of these patients had pre-existing ILD and received first-line chemotherapy. The clinical characteristics, treatment outcome, and survival of these patients were retrospectively reviewed using data obtained from

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Key Words: Interstitial lung disease, small cell lung cancer, chemotherapy, cisplatin, carboplatin, etoposide, acute exacerbation.

0250-7005/2013 $2.00+.40

Table I. *Patients' characteristics.*

Characteristic	N	%
Number of patients	52	
Age (years)		
Median (range)	71 (50-85)	
Gender		
Male	50	96
Female	2	4
Performance status		
0/1	9/37	88
2/3	5/1	12
Smoking status		
Never smoker	0	0
Current/former	25/27	100
Brinkman index median (range)	1050 (315-2940)	
Clinical stage		
Limited disease	29	56
Extensive disease	23	44

Table II. *Treatment outcome of first-line chemotherapy in 52 patients with small cell lung cancer with pre-existing Interstitial Lung Disease.*

	N	%
Number of patients	52	
First-line chemotherapy regimen		
Carboplatin plus etoposide	22	42
Cisplatin plus etoposide	30	58
Number of cycles (1/2/3/4)	8/6/6/32	15/12/12/61
Objective response		
CR	1	
PR	35	
SD	9	
PD	3	
NE	4	
Overall response rate (LD/ED)		72/65

CR, Complete response; PR, partial response; SD, stable disease; PD, progressive disease; NE, not evaluable; LD, limited disease; ED, extensive disease.

their medical records. The patients were staged according to the staging system of the Veterans Administration Lung Cancer Study Group as limited disease (LD) or extensive disease (ED) (9). In this study, two independent pulmonologists (T.Y. and K.Y.) who had no knowledge of the patients' outcome diagnosed pre-existing lung conditions based on pre-treatment chest computed tomography (CT) findings obtained before first-line chemotherapy. Pre-treatment conventional CT or high-resolution CT (HRCT) films of the chest were used in our analysis. Pre-existing ILD was diagnosed when diffuse ground-glass opacity, peripheral reticular opacity, and consolidation without segmental distribution and a honeycomb pattern were detected in bilateral lung fields on the chest X-ray and CT findings. The acute exacerbation of ILD was diagnosed based on the chest X-ray and/or CT findings, which showed newly-developed diffuse pulmonary opacities, physical findings, and serum levels of markers of damaged pneumocytes [*i.e.* lactate dehydrogenase (LDH), C-reactive protein (CRP), Krebs von den Lungen-6 (KL-6)] and the lack of a response to antibiotics. Patients with pulmonary infection, pneumothorax, pulmonary embolism, or heart failure were excluded.

The objective tumor response was assessed according to the Response Evaluation Criteria Solid Tumor (RECIST) (10). The objective response rate (ORR) was calculated as the total percentage of patients with a complete response (CR) or a partial response (PR). Toxicity was graded using the Common Terminology Criteria for Adverse Events (CTCAE), ver. 3.0 (11). A univariate analysis was performed to identify risk factors for the acute exacerbation of ILD in patients with SCLC with pre-existing ILD. All the variables were analyzed using the Fisher's exact test. Multivariate analyses were performed using logistic regression. A clinical evaluation of progression-free survival (PFS) was measured from the start of the first-line chemotherapy to the identifiable time for progression. The overall survival (OS) was measured as the period from the start of first-line chemotherapy until death from all causes. The PFS and OS were plotted using the Kaplan-Meier method. All the p-values were two-sided, and a level of 5% was considered statistically significant, unless otherwise specified.

Results

Patients' characteristics. The pre-treatment characteristics of the patients are shown in Table I. The median age at the time of first-line chemotherapy for SCLC was 71 years (range=50-85 years), 96% of them were men, and 88% had an Eastern Cooperative Oncology Group performance status (PS) of 0 or 1. All the patients were current or former smokers. None of the patients had histologically-confirmed interstitial pneumonia. Overall, 56% of the patients had LD and 44% had ED. As the first-line chemotherapy, 22 (42%) out of the 52 patients received carboplatin plus etoposide, and 30 (58%) patients received cisplatin plus etoposide (every 3 to 4 weeks). In the three cases, radiation therapy was performed after four cycles of chemotherapy. After progression, 33 patients received second-line chemotherapy. Subsequent chemotherapy regimens were amrubicin in 17 patients, cisplatin plus irinotecan in nine, irinotecan in six, carboplatin plus etoposide in five, topotecan in four, the combination of irinotecan, cisplatin plus etoposide in two, and carboplatin plus paclitaxel in one patient.

Treatment efficacy. Table II summarizes the treatment outcome of first-line chemotherapy. Regarding treatment delivery, the median number of administered cycles was four (range=1-4). Overall, 32 (61%) of the patients completed all four of the planned cycles. The treatment was discontinued because of progressive disease (PD) in seven patients, toxicity in nine, and other reasons in four patients. The ORR was 69% (72% in LD and 65% in ED, respectively), comprising of one CR and 35 PR. The response was not evaluable in four patients because of death before the first

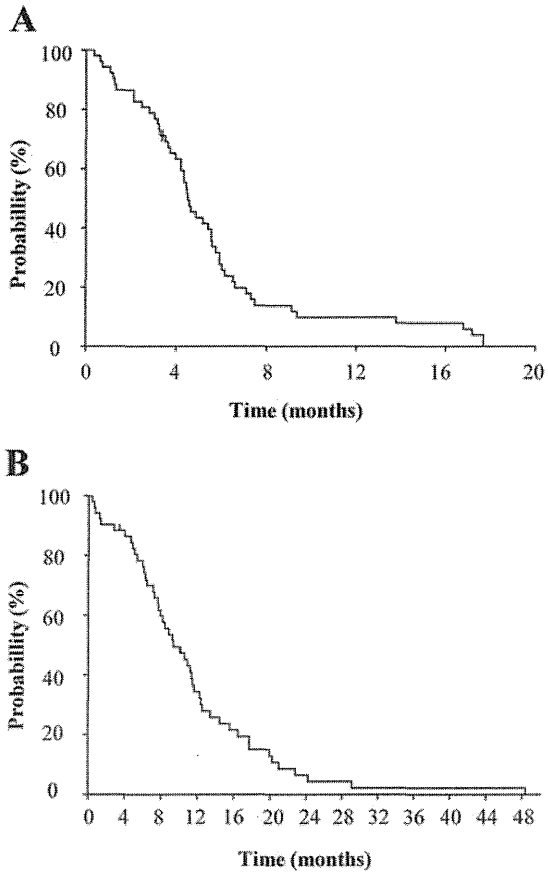

Figure 1. *Progression-free survival (PFS) (A) and overall survival (OS) (B) of patients with SCLC with Interstitial Lung Disease who received platinum agents plus etoposide as first-line chemotherapy. The vertical bars indicate the censored cases at the cut-off date. The median PFS, median OS, and 1-year survival times were 4.5 months [5.4 months, limited disease (LD) stage; 3.7 months, extensive disease (ED) stage], 9.4 months (10.6 months, LD stage; 8.2 months, ED stage), and 32%, respectively.*

tumor response evaluation. The median PFS after first-line chemotherapy and the median OS were 4.5 months (5.4 months, LD stage; 3.7 months, ED stage) (Figure 1A) and 9.4 months (10.6 months, LD stage; 8.2 months, ED stage), respectively (Figure 1B). Regarding the PFS and OS, the differences between the LD and ED stages were not statistically significant.

Incidence of acute exacerbation of ILD. Only one patient (2%) developed an acute exacerbation of ILD during first-line chemotherapy (carboplatin plus etoposide). During second- or third-line chemotherapy, five patients developed acute exacerbation of ILD. The regimens immediately before the development of the acute exacerbation of ILD were amrubicin in three patients, a combination of irinotecan, cisplatin, plus etoposide in one, and topotecan in one patient. The characteristics of all six patients with acute exacerbations of ILD, are listed in Table III. All the patients were smokers and men with a good PS before chemotherapy. The median time from the last administration of chemotherapy to the development of the acute exacerbation of ILD was 37 days. Although all the patients with acute exacerbation of ILD were treated using steroids, three out of the six patients did not improve and died. The results of univariate analyses of risk factors (age, sex, Brinkman index, LDH levels, and PS) for the acute exacerbation of ILD are listed in Table IV. No significant risk factors for acute exacerbation of ILD were identified. The results of the multivariate analysis for the acute exacerbation also showed that none of the variables were significant.

Discussion

In our study, the results for the 52 patients with SCLC with pre-existing ILD indicated that the combination of platinum agents plus etoposide as first-line chemotherapy yielded an ORR of 69%, a median PFS of 4.5 months, and a median OS of 9.4 months. Although directly comparable historical control data were not available, the observed efficacy in our study was the same as the results of two previous randomized phase III trials with platinum agents plus etoposide for ED stage patients with SCLC [Japan Clinical Oncology Group (JCOG) 9511: ORR=67.5%; PFS=4.8 months; median OS=9.4 months; and JCOG 9702: ORR=73%; PFS=5.2 months; median OS=10.6 months] (3, 4). Furthermore, the incidence of acute exacerbation of ILD during first-line chemotherapy observed in our study, was 2% (1/52). The combination of platinum agents plus etoposide seems to be effective and tolerable as a first-line chemotherapy for patients with SCLC with preexisting ILD.

The incidence of lung cancer is reported to be higher in patients with ILD than in patients without (8). In patients with lung cancer, pre-existing ILD has been reported to be a risk factor for the development of anticancer agent-associated acute exacerbation of ILD, which is a fatal complication of treatment. There are some reports regarding the safety and efficacy of chemotherapy for advanced or recurrent NSCLC with pre-existing ILD (6, 12, 13), and the incidence of acute exacerbation of ILD in NSCLC is reported to range from 20% to 24% in Japan, although the chemotherapeutic regimens that were administered were not the same (14, 15). Minegishi *et al.* reported the results of feasibility study for carboplatin plus etoposide in 17 SCLC patients with idiopathic interstitial pneumonias (IIPs) (16). The results indicated that the acute exacerbation of IIP occurred in one (5.9%) out of the 17 patients, with a median PFS of 5.5 months and a median OS of 8.7 months. However, that study was limited in that it included a small number of patients. It

Table III. *Summary of date for six patients who developed acute exacerbation of Interstitial Lung Disease.*

No.	Age (years)	Gender	PS	BI index	Prior chemotherapy	Time to AE after prior chemotherapy	Initial manifestations	AE status	Time to death after last chemotherapy (days)
1	71	Male	1	940	Carboplatin, Etoposide (1st line)	5 (day 5 in cycle 1)	Dyspnea	Died	19
2	53	Male	2	1490	Amrubicin (3rd line)	17 (day 17 in cycle 1)	Dyspnea, fever	Died	30
3	70	Male	1	1150	Cisplatin, Etoposide, Irinotecan (2nd line)	140 (day 93 in cycle 3)	Dyspnea	Died	123
4	50	Male	1	330	Topotecan (2nd line)	52 (day 24 in cycle 2)	Dyspnea	Improved	–
5	63	Male	1	960	Amrubicin (2nd line)	23 (day 23 in cycle 1)	Dyspnea, fever	Improved	–
6	62	Male	1	620	Amrubicin (3rd line)	73 (day 17 in cycle 3)	Dyspnea	Improved	–

PS, Performance status; BI, Brinkman index; AE, acute exacerbation.

was also unclear whether chemotherapy regimens such as platinum agents plus etoposide, which is the most frequently used regimen worldwide (4, 17), were feasible in patients with SCLC, with pre-existing ILD at the time of the start of our study.

In our study, acute exacerbation of ILD during second- or third-line chemotherapy occurred in five (16%) out of the 33 patients who received subsequent chemotherapy, compared with 2% (1/52) of the patients who received platinum agents plus etoposide as first-line chemotherapy. Previous reports have also shown that second-line chemotherapy has a high frequency and risk of the acute exacerbation of ILD, consistent with the results of the present study (15, 16). We speculated that the difference in the incidence of acute exacerbation of ILD between the first-line and subsequent chemotherapy regimens can be accounted for by some of the effective agents used for refractory SCLC, such as amrubicin and irinotecan, which are reportedly associated with a high incidence of acute exacerbation in patients with pre-existing ILD (18, 19).

Our study has a major limitation in that the diagnosis of acute exacerbation of ILD was not based on pathological findings but only on results of chest CT findings and the clinical course. We cannot completely exclude the possibility that the patients had developed lymphangitic carcinomatosis or some other disease, rather than acute exacerbation of ILD. However, pathological findings for the diagnosis of acute exacerbation of ILD are difficult to obtain. Therefore, we diagnosed acute exacerbation of ILD based on clinical and radiographic findings that were consistent with drug-induced ILD. Moreover, the pathological diagnosis of ILD using an open lung biopsy before treatment is extremely difficult and impractical, since chemotherapy should be started as soon as possible after the diagnosis of SCLC, which is characterized by rapid growth and widespread metastatic disease. We consider that the diagnosis of pre-existing ILD and the acute exacerbation of ILD based on clinical and radiological findings is appropriate in clinical practice.

Table IV. *Relationship between clinical variables and acute exacerbation of Interstitial Lung Disease.*

	No. of patients	Incidence of AE (%)	*p*-Value
Total	52	12	
Age			
<70 years	18	22	0.17
≥70 years	34	6	
Gender			
Male	50	12	>0.99
Female	2	0	
PS			
0/1	46	0	
2/3	6	13	
Smoking index			
<1000	21	14	0.68
≥1000	31	10	
LDH			
Normal	30	7	0.38
High (more than upper limit of normal)	22	18	

PS, Performance status; LDH, lactate dehydrogenase.

Our findings indicated that the combination of platinum agents plus etoposide is feasible and effective for the treatment of patients with SCLC with pre-existing ILD. A further large study is warranted to enable definitive conclusions to be drawn.

Acknowledgements

This work was supported in part by a Grant-in-Aid for Cancer Research from the Ministry of Health, Labour and Welfare of Japan.

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Received December 17, 2012
Revised January 29, 2013
Accepted January 30, 2013

J Cancer Res Clin Oncol (2013) 139:1691–1700
DOI 10.1007/s00432-013-1495-0

ORIGINAL PAPER

Solid predominant histology predicts EGFR tyrosine kinase inhibitor response in patients with EGFR mutation-positive lung adenocarcinoma

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Received: 18 March 2013 / Accepted: 9 August 2013 / Published online: 22 August 2013

Abstract
Background The efficacy of epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKIs) differs in patients with lung adenocarcinoma harboring *EGFR*-activating mutations. Although lung adenocarcinoma with *EGFR*-activating mutations has heterogeneous morphologic features, the predictive role of histologic subtype of lung adenocarcinoma with regard to the effectiveness of EGFR-TKIs in patients with *EGFR*-activating mutations has not been well defined.
Methods Among 134 postoperative recurrence patients with lung adenocarcinoma harboring *EGFR*-activating mutation (L858R or exon 19 deletion) treated with EGFR-TKIs, we retrospectively analyzed 61 patients treated with EGFR-TKIs as first-line chemotherapy. All the tumors were classified according to the new histologic classification proposed by the International Association for the Study of Lung Cancer (IASLC), American Thoracic Society (ATS), and European Respiratory Society (ERS) into the following subtypes: lepidic, papillary, acinar, micropapillary, or solid predominant subtype. We evaluated the correlation between the histologic subtype and the clinical efficacy of EGFR-TKIs.
Results In overall response rate, adenocarcinoma with solid predominant subtype is significantly worse than with non-solid predominant subtype (61 vs. 88 %, $P = 0.03$). The median progression-free survival (PFS) and overall survival after EGFR-TKI treatment were significantly shorter for the patients with solid predominant subtype than for those with non-solid predominant subtype (median PFS of 7.7 vs. 13.5 months, $P = 0.002$, and median OS of 21.5 vs. 31.0 months, $P = 0.028$).
Conclusions This study indicated that among patients with lung adenocarcinoma harboring activating *EGFR* mutations treated with EGFR-TKIs, solid predominant subtype according to IASLC/ATS/ERS classification is a response predictor for EGFR-TKI.

Keywords Lung adenocarcinoma · Epidermal growth factor receptor mutation · Epidermal growth factor receptor tyrosine kinase inhibitor · Solid predominant subtype

Abbreviations

EGFR	Epidermal growth factor receptor
TKI	Tyrosine kinase inhibitor
NSCLC	Non-small cell lung cancer
PFS	Progression-free survival
IASLC	International Association for the Study of Lung Cancer
ATS	American Thoracic Society
ERS	European Respiratory Society
RECIST	Response evaluation criteria solid tumor criteria
ORR	Objective response rate
OS	Overall survival

Electronic supplementary material The online version of this article (doi:10.1007/s00432-013-1495-0) contains supplementary material, which is available to authorized users.

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HR	Hazard ratio
CI	Confidence interval
HGF	Hepatocyte growth factor

Introduction

Epidermal growth factor receptor (EGFR) mutations have recently been reported to be a predictive factor for the efficacy of EGFR tyrosine kinase inhibitors (EGFR-TKIs) in patients with advanced non-small cell lung cancer (NSCLC) (Mok et al. 2009; Mitsudomi et al. 2010; Maemondo et al. 2010). The *EGFR* mutation status is frequently associated with patient characteristics that have been previously shown to be correlated with a clinical response to EGFR-TKI treatment: nonsmokers, women, individuals with an Asian ethnic background, and adenocarcinoma histology (Lynch et al. 2004; Paez et al. 2004; Calvo and Baselga 2006; Toyooka et al. 2007). Most *EGFR* mutations are either short in-frame deletions in exon 19 or point mutations that result in a substitution of arginine for leucine at amino acid 858 (L858R) (Paez et al. 2004; Lynch et al. 2004; Kosaka et al. 2004). The EGFR-TKIs showed a significant prolongation of progression-free survival (PFS), compared with standard first-line cytotoxic chemotherapy, in NSCLC patients with *EGFR*-activating mutations, such as exon 19 deletions and L858R (Mok et al. 2009; Kosaka et al. 2004; Sica et al. 2010; Rosell et al. 2012). Patients with *EGFR* mutation have a significantly longer survival than those with wild-type *EGFR* when treated with EGFR-TKIs (Mitsudomi et al. 2005; Takano et al. 2005). However, the clinical efficacy of EGFR-TKIs differs among NSCLC patients harboring *EGFR* mutation, and most patients develop resistance to these drugs (Jackman et al. 2010; Herbst et al. 2008). Therefore, the differential response in patients with the same *EGFR* mutation status and acquired resistances to EGFR-TKIs are major problems in the management of *EGFR*-mutant lung cancer.

Among the histologic types of NSCLC, adenocarcinoma is well known to have heterogeneous morphologic features and to have diverse properties in the presence of *EGFR* mutations. The 2011 International Association for the Study of Lung Cancer (IASLC)/American Thoracic Society (ATS)/European Respiratory Society (ERS) has proposed a new classification system for invasive adenocarcinoma based on the predominant subtype using a method called comprehensive histologic subtyping to estimate semiquantitatively the percentages of the subtypes that are present within the tumors: lepidic, papillary, acinar, micropapillary, and solid patterns in surgically resection specimens (Travis et al. 2011). These differences in the morphology of adenocarcinoma might reflect biologic behaviors (Sica et al. 2010; Motoi et al. 2008; Russell et al. 2011; Warth et al. 2012).

To our knowledge, no evidence has been reported suggesting that histologic features have a predictive role associated with the effectiveness of a specific treatment for lung adenocarcinoma patients, especially the use of EGFR-TKIs for lung adenocarcinoma patients harboring *EGFR* mutation. Therefore, we investigated the association between the predominant subtype of surgically resected specimens and the response to EGFR-TKIs in lung adenocarcinoma patients with postoperative recurrences harboring an *EGFR*-activating mutation. We also analyzed the correlation between the histologic subtype of small biopsy specimens and the response to EGFR-TKIs in advanced lung adenocarcinoma patients harboring an *EGFR*-activating mutation.

Materials and methods

Patients

The first cohort contained 134 postoperative recurrence patients with lung adenocarcinoma harboring an *EGFR*-activating mutation (L858R or exon 19 deletion) treated with EGFR-TKI (250 mg/day of gefitinib, administered orally) at our institution between January 2002 and December 2011. The second cohort contained 71 patients with clinical stage IIIB or IV lung adenocarcinoma harboring an *EGFR*-activating mutation treated with EGFR-TKIs (250 mg/day of gefitinib or 150 mg/day of erlotinib, administered orally) between January 2009 and December 2011. To evaluate the association between clinicopathological features and the response to EGFR-TKI in this study, we defined assessable patients as follows: presence of evaluable lesions according to the Response Evaluation Criteria Solid Tumor criteria (RECIST) version 1.1 (Eisenhauer et al. 2009), presence of sufficient tissue for histologic evaluation, and no history of chemotherapy without adjuvant chemotherapy. Of 134 patients with postoperative recurrences, we selected 61 assessable patients treated with EGFR-TKIs as first-line chemotherapy who met these criteria. In a second cohort, 41 assessable patients treated with EGFR-TKIs as first-line chemotherapy were included.

Histologic evaluation of surgically resected specimens

All surgical specimens were fixed with 10 % formalin or absolute methanol and embedded in paraffin. Serial 4-μm sections were stained with hematoxylin and eosin. Two observers (T.Y. and G.I.) who were unaware of the clinical

data independently reviewed all the pathological slides. The histologic diagnosis was based on the 2004 World Health Organization histologic classification. In addition, surgically resected specimens of lung adenocarcinomas were classified according to the 2011 IASLC/ATS/ERS International Multidisciplinary Classification of lung adenocarcinomas into the following subtypes: lepidic predominant subtype (Fig. 1a), acinar predominant subtype (Fig. 1b), papillary predominant subtype (Fig. 1c), micropapillary predominant subtype, and solid predominant subtype (Fig. 1d). Histologic subtyping was performed in the primary tumor in a semiquantitative manner, with the percentage of five possible histologic subtypes quantified in 10 % increments, totaling 100 % per tumor. The predominant subtype was defined as the histologic component that comprised the largest percentage among the components. The histologic findings for the small biopsy samples diagnosed as adenocarcinoma were classified into five patterns according to the morphological criteria of surgically resected specimens: lepidic (Fig. 2a), acinar (Fig. 2b), papillary (Fig. 2c), solid (Fig. 2d), and micropapillary subtype.

Analysis of EGFR status and EGFR-TKIs response

We evaluated two types of activating *EGFR* mutations (deletions in exon 19 and L858R) using either the direct sequencing method (SRL, Tokyo, Japan), the peptide nucleic acid-locked nucleic acid (PNA-LNA) PCR clamp method (Chemical Medience, Tokyo, Japan), or a PCR-Invader Assay (BML, Tokyo, Japan). The objective response rate (ORR) was calculated as the total percentage of patients with a complete response (CR) or a partial response (PR). A clinical evaluation of PFS was measured from the start of the first-line chemotherapy to the earliest identifiable sign of disease progression as determined using CT or MRI imaging using the RECIST or any cause of death. The overall survival (OS) was measured as the period from the start of first-line chemotherapy until death from any cause.

Statistical analysis

All the statistical analyses were performed using JMP for Windows version 9 statistical software package (SAS

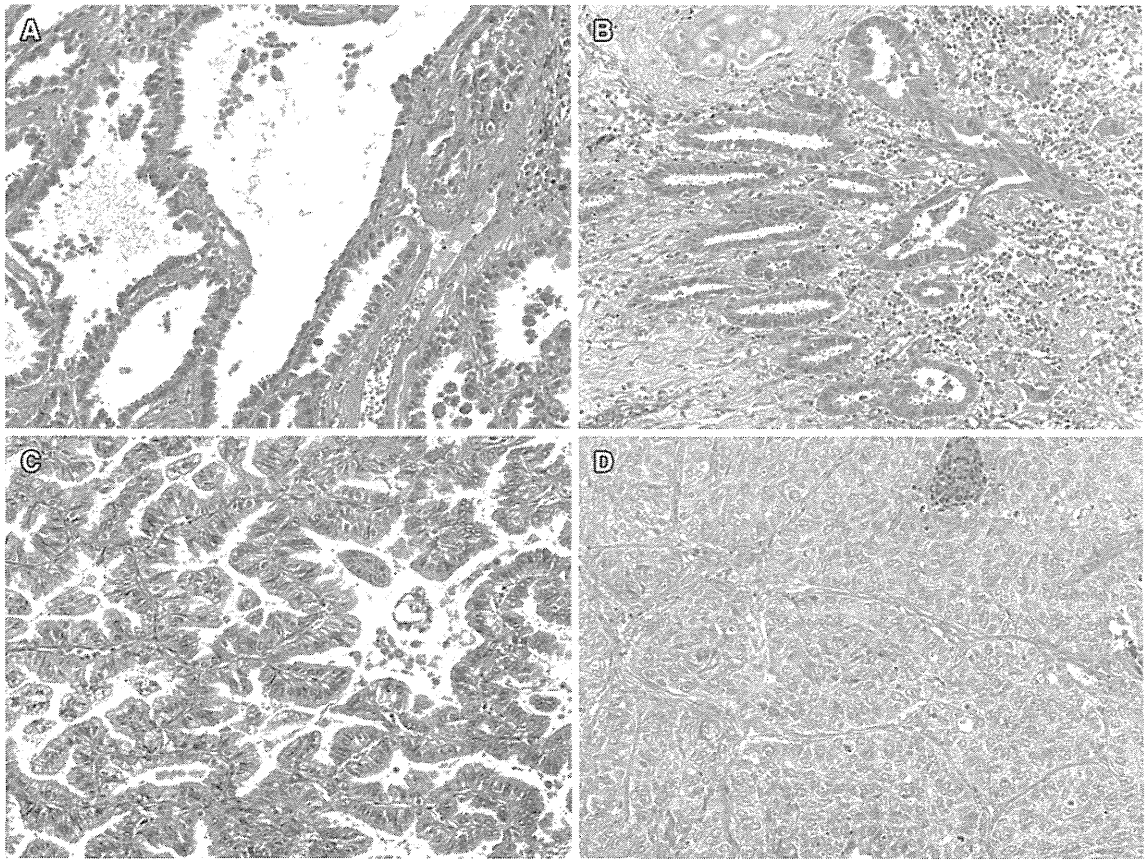

Fig. 1 Microscopic appearance of the 4 histologic subtypes. **a** Lepidic pattern, **b** acinar pattern, **c** papillary pattern, **d** solid pattern (hematoxylin-eosin, (**a**, **b**, **c**, and **d**), original magnifications: objective lens ×10)

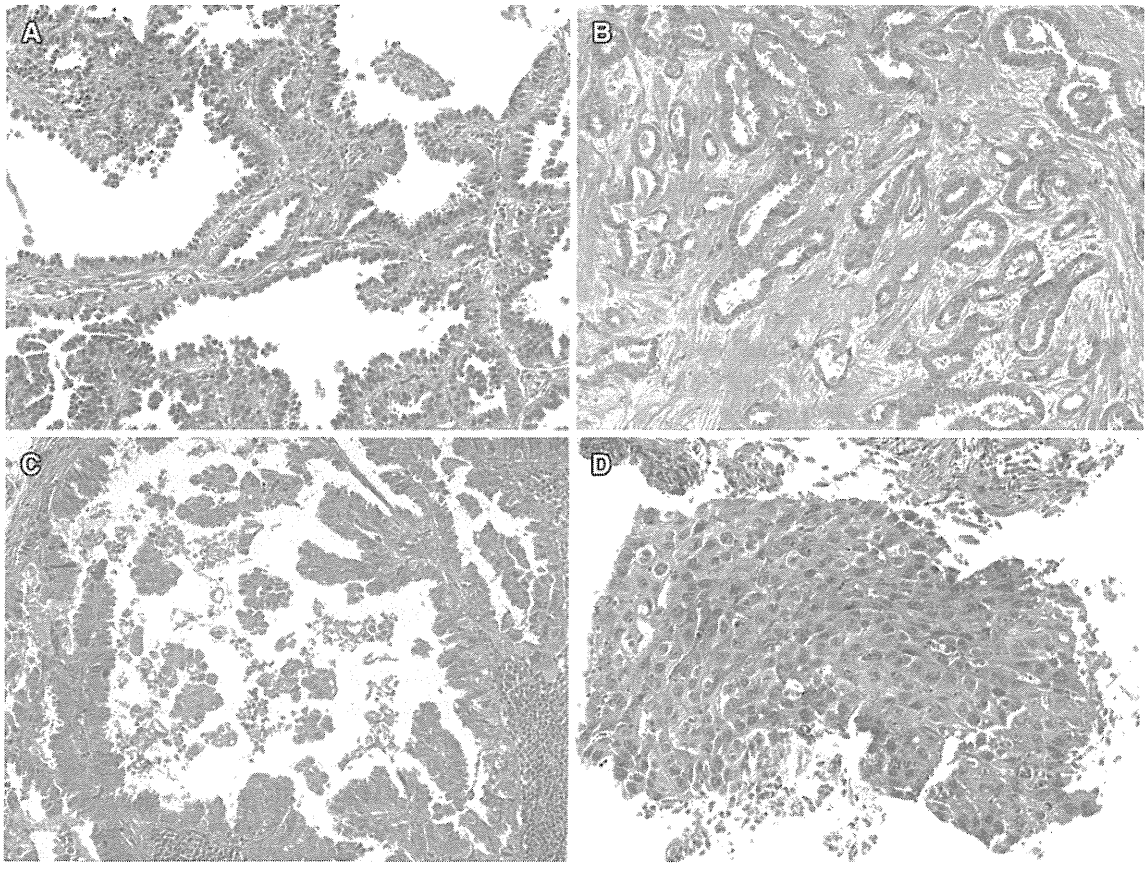

Fig. 2 Microscopic appearance of the 4 histologic subtypes in biopsy specimen. **a** Lepidic pattern, **b** acinar pattern, **c** papillary pattern, **d** solid pattern (hematoxylin-eosin, (**a**, **b**, **c**, and **d**), original magnifications: objective lens ×10)

Institute, NC, USA). The chi-square test or the Fisher exact test was used to determine the statistical significance of differences between the two groups. The PFS and OS were estimated using the Kaplan–Meier method, and differences in the variables were calculated using the log-rank test. Cox proportional hazard model was used to adjust the treatment effect for the baseline factors such as age, gender, performance status (PS), smoking status, *EGFR* mutation types, adjuvant chemotherapy, and pathological factors associated with prognosis and histologic subtype. All the *P* values were two sided, and a level of 5 % was considered statistically significant, unless otherwise specified. This study was approved by the Institutional Review Boards of the National Cancer Center.

Results

Patient characteristics with postoperative recurrence

The patient characteristics of the first cohort are listed in Table 1. An exon 19 deletion and an L858R point mutation were detected in 29 and 32 of the patients, respectively. All the patients received gefitinib as first-line chemotherapy. The pathologic characteristics of the surgical specimens are summarized in Table 2. The majority of histologic subtypes were composed of the papillary predominant subtype (31 cases; 51 %), followed in frequency by the solid predominant subtype (18 cases; 29 %), the acinar predominant subtype (6 cases; 10 %), and the lepidic predominant subtype (6 cases; 10 %). A micropapillary predominant subtype was not detected among this cohort.

ORR of postoperative recurrent patients with each predominant histologic subtype

In response to treatment, 49 of 61 patients had PR, 7 patients had SD, and 5 patients had PD; the ORR was 80 % in all patients (Table 1), 67 % in lepidic predominant subtype, 90 % in papillary predominant subtype, 100 % in acinar predominant subtype, and 61 % in solid predominant subtype. The response rate did not differ significantly between patients with the lepidic and non-lepidic (67 vs. 82 %, $P = 0.59$), papillary and non-papillary (90 vs. 70 %, $P = 0.06$), acinar and non-acinar (100 vs. 78 %, $P = 0.59$), but patients with solid predominant subtype had

Table 1 Patients characteristics ($N = 61$)

Characteristic	No. of cases	%
All patients	61	
Age (years)		
Median (range)	68 (42–85)	
Gender		
Male	28	45
Female	33	55
Performance status		
0/1	54	89
2/3	7	11
Smoking status		
Never smoker	37	61
Former smoker	18	29
Current smoker	6	10
EGFR mutation status		
Exon 19 deletion	29	48
L858R	32	52
Adjuvant chemotherapy		
Yes	24	39
No	37	61
First-line EGFR-TKI		
Gefitinib	61	100
Objective response		
CR	0	0
PR	49	80
SD	7	12
PD	5	8
Overall response rate		80

EGFR epidermal growth factor receptor, *TKI* tyrosine kinase inhibitor, *CR* complete response, *PR* partial response, *SD* stable disease, *PD* progressive disease

Table 2 The pathological characteristics of primary tumor ($N = 61$)

Characteristics	No. of cases	%
Histologic subtype		
Lepidic predominant	6	10
Acinar predominant	6	10
Papillary predominant	31	51
Solid predominant	18	29
Pathological stage		
IA	8	13
IB	10	16
IIA	4	7
IIB	2	3
IIIA	28	46
IIIB	5	8
IV	4	7
Lymphatic permeation		
Positive	27	44
Negative	34	56
Vascular invasion		
Positive	43	70
Negative	18	30
Pleural invasion		
Positive	31	51
Negative	30	49
Pulmonary metastases		
Positive	12	20
Negative	49	80

worse response rate than non-solid predominant subtype (61 vs. 88 %, $P = 0.03$, Table 3).

Association between predominant histologic subtype and PFS after EGFR-TKI in patients with postoperative recurrence

At the time of the analysis, the median follow-up time was 22 months (range 1–56 months). The median PFS after gefitinib treatment was 12 months in all the patients with postoperative recurrence. The median PFS in patients with solid predominant subtype was 7.7 months (95 % confidence interval (CI) 4.1–11.7), as opposed to that in those with lepidic, papillary, or acinar predominant subtype of adenocarcinoma, in whom it was 9.4 months (95 % CI 0.7–43.9), 13.3 months (95 % CI 11.6–16.4), and 18.6 months (95 % CI 9.4–23.7), respectively. No statistically significant difference in the PFS was observed between the lepidic and non-lepidic predominant subtypes (median of 9.4 vs. 12.1 months (95 % CI 11.3–14.1), $P = 0.597$), the papillary and non-papillary predominant subtypes (median of 13.3 vs. 10 months (95 % CI 6.3–14.1), $P = 0.118$), or the acinar and non-acinar predominant subtypes (median of 18.6 vs. 11.7 months (95 % CI 9.8–13.5), $P = 0.639$). However, the patients with the solid predominant subtype showed significantly shorter PFS than those with non-solid predominant subtype (median of 7.7 vs. 13.5 months (95 % CI 11.6–17.3], $P = 0.002$, Fig. 3a). In addition, the overall survival of the solid predominant subtype patients was shorter than that of the non-solid predominant subtype patients (median of 21.5 months (95 % CI 6.9–32.1) vs. 31.0 months (95 % CI 19.8–43.9), $P = 0.028$, Fig. 3b).

Predictive factors of the PFS after EGFR-TKIs in patients with postoperative recurrence

The results of the univariate and multivariate analyses determining predictive factors of the PFS after EGFR-TKI as first-line chemotherapy are shown in Table 4. The

univariate and multivariate analysis revealed that solid predominant subtype was significantly predictive factor associated with the PFS (solid predominant subtype: hazard ratio (HR), 3.973; 95 % CI 1.892–8.294; P <0.001).

Association between histologic pattern in small biopsy samples and response after EGFR-TKI in patients with advanced lung adenocarcinoma

The patient characteristics with advanced lung adenocarcinoma are shown in Table 5. Exon 19 deletions and L858R point mutations were detected in 19 and 22 patients, respectively. The histologic subtype was lepidic in 3 patients, acinar in 17 patients, papillary in 8 patients, and solid subtype in 13. The ORR and median PFS were 73 % and 8.8 months in all the patients with advanced lung adenocarcinoma. Based on the result of postoperative recurrent disease, all the tumors were divided into two subtypes, solid subtype, and non-solid subtype. The ORR and median PFS were significantly worse in patients with adenocarcinoma with solid subtype than in those with non-solid subtype (ORR; 50 vs. 86 %, $P = 0.04$, median PFS; 5.4 months (95 % CI 1.5–10) vs. 10.0 months (95 % CI 8.8–16.9), $P = 0.006$) (Fig. 4). The median overall survival had not been reached for either solid pattern or non-solid pattern groups (data not shown). The univariate and multivariate analysis showed that solid subtype in small biopsy samples was the only predictive factor of the response duration with EGFR-TKI (solid subtype: HR, 3.401; 95 % CI 1.381–8.135; $P = 0.009$) (Table 6).

Table 3 Response rate to EGFR-TKI in solid predominant subtype ($N = 18$) and non-solid predominant subtype ($N = 43$)

Objective response	Solid predominant subtype N (%)	Non-solid predominant subtype N (%)	P value
Overall response rate	61	88	0.03
PR	11 (61)	38 (88)	
SD	4 (22)	3 (7)	
PD	3 (17)	2 (5)	

PR partial response, *SD* stable disease, *PD* progressive disease

Discussion

In the current study, we found that lung adenocarcinoma with solid predominant subtype was significantly associated with a shorter PFS after EGFR-TKI treatment in patients with *EGFR*-activating mutations. This study is the first to report that the histologic subtype is associated with the duration of the response to EGFR-TKIs.

A few reports have shown predictive markers of the duration of the response to EGFR-TKIs in patients with *EGFR* mutations. Azuma et al. reported that a high score calculated from both the staining intensity and the proportion of the tumor tissue with mutant EGFR expression in an immunohistochemical analysis with mutant-specific antibodies was associated with a longer PFS in postoperative recurrence patients with *EGFR* mutations (Azuma et al. 2012). Yano et al. showed that high-level hepatocyte growth factor (HGF) expression using immunohistochemistry was more strongly associated with intrinsic and acquired EGFR-TKI resistance than *EGFR* T790M secondary mutation in *EGFR*-mutant lung cancer (Yamada et al. 2012). However, these reports included two intermingled patient groups containing both postoperative

Table 4 Impact of predictive factors on PFS of EGFR-TKI by univariate and multivariate analysis ($n = 61$)

Variables	Univariate analysis P value	Multivariate analysis		
		Hazard ratio	95 % CI	P value
Age (≥70)	0.952	0.528	0.253–1.084	0.082
Gender (male)	0.226	2.007	0.953–4.234	0.067
Performance status (≥2)	0.708	2.431	0.754–6.973	0.130
Smoking status (smoker)	0.810	0.803	0.387–1.658	0.552
Pathological stage (stage III, IV)	0.858	1.229	0.528–2.891	0.633
EGFR status (L858R)	0.337	1.781	0.896–3.539	0.099
Predominant subtype (solid predominant subtype)	0.002	3.973	1.892–8.294	<0.001
Lymphatic permeation (positive)	0.845	0.531	0.247–1.100	0.089
Vascular invasion (positive)	0.392	1.040	0.436–2.504	0.929
Pleural invasion (positive)	0.092	1.863	0.793–4.765	0.157
Pulmonary metastases (positive)	0.799	1.646	0.697–3.801	0.250
Adjuvant chemotherapy (yes)	0.686	0.558	0.266–1.187	0.128

CI confidence interval

Table 5 Patients characteristics with advanced lung adenocarcinoma ($N = 41$)

Characteristic	No. of cases	%
All patients	41	100
Age (years)		
Median (range)	66 (36–84)	
Gender		
Male	17	41
Female	24	59
Performance status		
0/1	35	85
2/3	6	15
Smoking status		
Never smoker	24	59
Former smoker	15	37
Current smoker	2	4
Clinical stage		
Stage IIIB	2	5
Stage IV	39	95
EGFR mutation status		
Exon 19 deletion	19	46
L858R	22	54
Biopsy methods		
Bronchoscopic biopsy	29	71
Fine needle biopsy	9	22
Pleural biopsy	3	7
First-line EGFR-TKI		
Gefitinib	36	88
Erlotinib	5	12
Histologic subtype		
Lepidic subtype	3	7
Acinar subtype	17	41
Papillary subtype	8	20
Solid subtype	13	32
Objective response		
CR	0	0
PR	30	73
SD	7	17
PD	2	5
NE	2	5
Overall response rate		73

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

recurrence patients and advanced lung cancer patients and were not limited to first-line chemotherapy.

Next, we evaluated whether solid patterns of small biopsy samples also had predictive impact on the response of EGFR-TKIs in advanced lung adenocarcinoma patients with *EGFR*-activating mutations. The ORR and median PFS were significantly worse in patients with adenocarcinoma with solid subtype than in those with non-solid subtype. Since small biopsy samples may not be representative of the total tumor (Travis et al. 2011), we compared the histologic subtype in small biopsy specimens and matched surgically resected samples in 122 patients (Supplemental Table 1). The sensitivity and specificity of solid subtype in small biopsy samples for the detection of solid predominant subtype were 84 and 90 %, respectively. Therefore, we think that the division into solid and non-solid subtype in small biopsy samples was reasonable, and solid subtype in small biopsy samples had negative predictive impact on the response of EGFR-TKIs in advanced lung adenocarcinoma patients.

The molecular mechanism responsible for the association between solid predominant subtype and shorter PFS after treatment with EGFR-TKIs as a first-line chemotherapy is unknown. To date, two possible mechanisms of EGFR-TKIs resistance, *EGFR* T790M secondary mutation (Kobayashi et al. 2005; Pao et al. 2005), and *MET* amplification (Cappuzzo et al. 2009; Engelman et al. 2007) have been confirmed. About half of all cancers that are resistant to EGFR-TKIs develop a secondary mutation in *EGFR* (T790M) (Bean et al. 2007; Kosaka et al. 2006), which is associated with a poor outcome of EGFR-TKI therapy (Maheswaran et al. 2008). Su et al. reported that the T790M mutation status before treatment, as assessed using a highly sensitive detection method, was an independent predictor of decreased PFS in NSCLC patients who received EGFR-TKI treatment (Su et al. 2012). In all the patients of present study, T790M mutation was not detected using general detection methods such as direct sequencing method, PNA-LNA PCR clamp, and PCR-Invader Assay. In future studies, an evaluation of the association between the histologic subtypes, such as solid predominant subtype, and the T790M status assessed using a highly sensitive detection method is needed.

On the other hand, about 15–20 % undergo amplification of the MET receptor tyrosine kinase, which activates downstream intracellular signaling independent of EGFR in patients with acquired resistant to EGFR-TKIs (Bean et al. 2007; Calvo and Baselga 2006; Turke et al. 2010). Tsuta et al. reported that high c-Met expression using immunohistochemistry and high *MET* gene amplification using bright-field in situ hybridization were more strongly associated with poorly differentiated adenocarcinomas, consistent with solid growth pattern, than with well-differentiated ones (Tsuta et al. 2012). More recently, HGF, a ligand of MET, was identified as a mechanism of EGFR-TKI resistance (Yano et al. 2008). HGF is produced by various stromal cells, especially fibroblasts (Birchmeier et al. 2003). Wang et al. revealed that stromal fibroblasts play a definitive role in acquired resistance to EGFR-TKIs through the binding of HGF to MET (Wang et al. 2009).

Table 6 Impact of predictive factors on PFS of EGFR-TKI in patients with advanced lung adenocarcinoma having a EGFR-activating mutation by univariate and multivariate analysis ($n = 41$)

Variable	Univariate analysis *P* value	Multivariate analysis		
		Hazard ratio	95 % CI	*P* value
Age (≥70)	0.167	0.480	0.175–1.123	0.093
Gender (male)	0.821	0.884	0.337–2.221	0.796
Performance status (≥2)	0.444	0.634	0.168–1.968	0.444
Smoking status (smoker)	0.225	1.579	0.614–4.215	0.344
Clinical stage (stage IV)	0.258	1.658	0.182–11.802	0.628
EGFR status (L858R)	0.779	1.835	0.714–4.752	0.206
Histologic pattern (solid subtype)	0.006	3.401	1.381–8.135	0.009

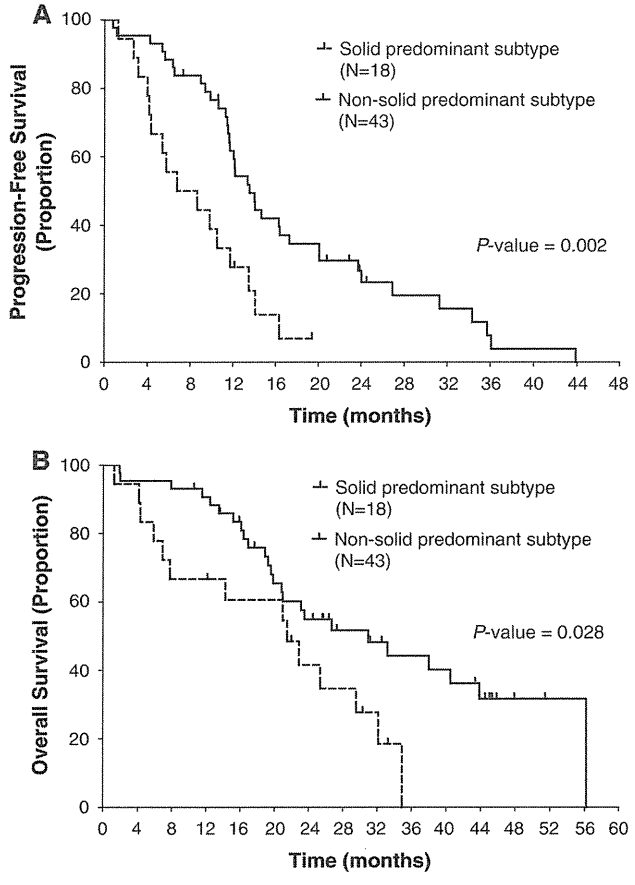

Fig. 3 **a** PFS of gefitinib as first-line chemotherapy in lung adenocarcinoma patients with postoperative recurrence harboring *EGFR*-activating mutation according to histologic predominant subtype (solid vs. non-solid) ($N = 61$), and **b** overall survival

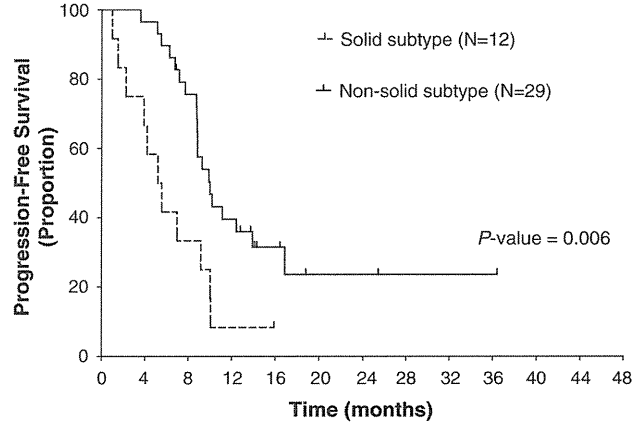

Fig. 4 PFS of EGFR-TKI as first-line chemotherapy in patients with advanced lung adenocarcinoma harboring *EGFR*-activating mutation according to histologic pattern (solid subtype vs. non-solid subtype) ($N = 41$)

It would also be possible to think that the tumor microenvironment created by both cancer cells with solid subtype morphology and non-cancerous cells, including cancer-associated fibroblasts, may play critical roles in the sensitivity to EGFR-TKIs.

Further studies are required to confirm the association between the histologic subtype and the response to EGFR-TKIs in validation populations, including multicenter, prospective studies. However, the present study is the first to show that a certain type of histology, solid predominant subtype, is a response predictor for EGFR-TKI. We believe that our data provide a novel insight for the clinical implications of the patients with *EGFR*-activating mutations.

Acknowledgments This work was supported by National Cancer Center Research and Development Fund (23-A-12 and 23-K-18), the Foundation for the Promotion of Cancer Research, 3rd-Term Comprehensive 10-Year Strategy for Cancer Control, Program for the Promotion of Fundamental Studies in Health Sciences of the National Institute of Biomedical Innovation, and JSPS KAKENHI (24659185).

Conflict of interest All authors declare no conflicts of interest.

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