P450 in the liver. CDHP increases the plasma concentration of 5-FU through competitive inhibition of dihydropyrimidine dehydrogenase, which catalyzes 5-FU catabolism. CDHP also attenuates the indirect cardiotoxic and neurotoxic effects of 5-FU by reducing the production of fluoro-β-alanine, the main catabolite of 5-FU. Oxo reduces the gastrointestinal toxicity of 5-FU. After its oral administration, Oxo becomes distributed selectively to the small and large intestine, where it inhibits the phosphorylation of 5-FU to fluoropyrimidine monophosphate catalyzed by orotate phosphoribosyltransferase within gastrointestinal mucosal cells, thereby reducing the incidence of diarrhea [4]. S-1 has shown promising antitumor activity as a single agent for the treatment of advanced NSCLC as well as a good safety profile with manageable toxicities [5]. Furthermore, we recently presented the results of a phase III trial showing that S-1 in combination with carboplatin is not less efficacious and is better tolerated than carboplatin–paclitaxel, a representative platinum-based doublet chemotherapy for first-line treatment of advanced NSCLC [6].

We have previously shown that the combination of S-1 and gefitinib has a synergistic antiproliferative effect on NSCLC cells regardless of the absence or presence of *EGFR* mutations and that this enhanced antitumor effect is mediated by gefitinib-induced down-regulation of thymidylate synthase, a major target of 5-FU [7]. The combination of S-1 and gefitinib also exerted a synergistic antitumor effect in gefitinib-resistant cells with *MET* amplification both in vitro and in vivo, suggesting that such combination therapy is a promising strategy to overcome gefitinib resistance [8]. On the basis of these preclinical data, we have performed a phase I trial to assess the safety–tolerability, pharmacokinetics, and antitumor efficacy of the combination of gefitinib and S-1 in patients with advanced adenocarcinoma of the lung.

Patients and methods

Patient selection

Eligible patients had a confirmed histological or cytological diagnosis of adenocarcinoma of the lung that was either recurrent or stage IIIB or IV; had failed at least one prior systemic anticancer regimen including one platinum-based regimen (up to two regimens allowed); had not previously received therapy with an EGFR-TKI or S-1; and had adequate organ function (hemoglobin level $\geq$9.0 g/dl, neutrophil count $\geq$1,500/mm^3, platelet count $\geq$100,000/mm^3, total bilirubin level $\leq$1.5 mg/dl, aspartate (AST) and alanine (ALT) aminotransferase levels of $\leq$100 IU/l, saturation of peripheral O_2 $\geq$90 %, serum creatinine concentration $\leq$1.2 mg/dl, and predicted creatinine clearance or 24-h creatinine clearance $\geq$60 ml/min as estimated by the Cockcroft and Gault formula [9]). The study protocol was approved by the institutional review board at each participating center, and the study was conducted in accordance with the guidelines of the Declaration of Helsinki. All patients provided written informed consent before study-related procedures were performed. This trial was registered at the UMIN Clinical Trials Registry (UMIN 000001594).

Study design

Patients received a fixed daily dose of gefitinib (250 mg) for an initial period of 14 days followed by continuous daily administration of gefitinib and the administration of S-1 for 14 consecutive days every 21 days until disease progression or development of intolerable toxicity. The dose level of S-1 was set at 40 mg/m^2 (level 0), 60 mg/m^2 (level 1), or 80 mg/m^2 (level 2), with the dose escalation following a traditional 3 + 3 phase I trial design. The dose escalation–reduction scheme was based on the occurrence of a drug-related dose-limiting toxicity (DLT) within the first treatment course. A DLT was defined as a toxicity occurring in cycle 1 that met one of the following criteria: neutropenia of grade 4 persisting for $\geq$7 days, febrile neutropenia, thrombocytopenia of grade 4, or a nonhematologic toxicity (with the exception of nausea, vomiting, or anorexia) of grade 3. A delay of >2 weeks in administering the second treatment cycle was also considered a DLT. The maximum tolerated dose (MTD) was defined as the highest dose level at which $\leq$33 % of the patients experienced a DLT during the first treatment cycle. After the MTD had been determined, the corresponding cohort was to be expanded to a maximum of 20 patients for a more complete assessment of the safety and tolerability of the dose level. At least 14 patients were to be treated at the recommended dose. The probability of adverse events (AEs) with an incidence of $\geq$20 % not being detected in any of the 14 patients was 4.4 %.

If a DLT was not observed in any of the first three patients in the first cohort (level 1), an escalated dose of S-1 (80 mg/m^2) was administered to the first three patients at level 2. If a DLT was observed in one or two of the first three patients, an additional three patients were enrolled to assess the tolerability of this dose level. If a DLT occurred in one or two of the six patients at level 1, the dose of S-1 was escalated (to 80 mg/m^2). If three or more of the six patients at level 1 experienced a DLT, additional patients were recruited at level 0. In addition to this dose escalation–reduction scheme, if the investigators and an independent data-monitoring committee agreed that additional patients were necessary to confirm the dose escalation–

reduction decision in cases in which two or more patients experienced DLTs that were not life-threatening and were reversible and manageable with or without medication, then the entry of additional patients at that dose level was allowed.

Pharmacokinetics

The plasma pharmacokinetics of single-agent and combination treatments were investigated in the dose-escalation phase of the study in order to assess the potential for interaction between gefitinib and S-1. The pharmacokinetics of gefitinib were evaluated for 2 days (day 14 of the run-in period of administration of gefitinib alone and day 1 of combination therapy with gefitinib and S-1), and those of S-1 were examined on the first day of combination therapy with gefitinib and S-1. The plasma concentration of gefitinib was measured by Shin Nippon Biomedical Laboratories (Wakayama, Japan). The plasma concentrations of S-1 components (FT, CDHP, and Oxo) and 5-FU were measured by FALCO Biosystems (Kyoto, Japan). All concentrations were determined with the use of liquid chromatography and tandem mass spectrometry [10].

Efficacy measures

All patients underwent a comprehensive baseline assessment including clinical laboratory tests and imaging studies. Toxicity evaluations were based on the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE) version 3.0. Computed tomography scans were obtained every 6 weeks for the first 3 months and every 2 months thereafter. Response was evaluated according to RECIST 1.0. Progression-free survival (PFS) was calculated from the first day of combination therapy with gefitinib and S-1 until the first occurrence of progression, death from any cause, or last follow-up. Overall survival (OS) was calculated from the first day of the combination therapy until death from any cause or the date of last contact. The probability of survival as a function of time was estimated with the Kaplan–Meier method.

Results

Patient characteristics

Between July 2008 and April 2010, twenty patients with advanced adenocarcinoma of the lung were enrolled in the study at the three participating centers. The characteristics of the 20 study patients are summarized in Table 1. The patients included 12 (60 %) women and 10 (50 %) never-smokers. All had adenocarcinoma, and 8 (40 %) had disease of stage IV. The median age was 61 years, with a range of 51–70 years. Thirteen (65 %) of the 20 patients had received one prior chemotherapy regimen, whereas 7 individuals (35 %) had been treated with two prior regimens. Samples from 15 patients were available for *EGFR* mutational analysis, with such mutations being detected in 9 patients [the L858R point mutation in 5 patients (56 %) and exon-19 deletions in 4 patients (44 %)].

Determination of recommended dose

No DLTs were apparent for the first three patients treated at dose level 1, and so three patients were entered at dose level 2 (Table 2). Two of these latter three patients experienced a DLT [alkaline phosphatase (ALP) increase in grade 3 in one patient; AST and ALT increases in grade 3 in the other] in the first cycle, and an additional three patients were therefore treated at dose level 2. None of these three additional patients experienced a DLT. According to the protocol definition, dose level 2 was determined as the recommended dose, and an additional 11 patients were assigned to this level. A total of 17 patients were therefore treated at dose level 2.

Table 1 Characteristics of the study patients ($n = 20$), the median age of whom was 61 years (range 51–70 years)

Characteristics	No. of patients
Sex	
Male	8 (40 %)
Female	12 (60 %)
Performance status (ECOG)	
0	4 (20 %)
1	16 (80 %)
Disease stage	
IIIB	8 (40 %)
IV	8 (40 %)
Postoperative recurrence	4 (20 %)
No. of previous chemotherapies	
1	13 (65 %)
2	7 (35 %)
EGFR mutation	
Positive (L858R, exon-19 deletion)	9 (45 %)
Negative	6 (30 %)
Unknown (not examined)	5 (25 %)
Smoking history (pack-years)	
0	10 (50 %)
1–19	4 (20 %)
≥20	6 (30 %)

ECOG Eastern Cooperative Oncology Group

Table 2 Dose-escalation scheme and dose-limiting toxicities (DLTs)

Level	Gefitinib (mg/body)	S-1 (mg/m^2)	No. of patients		Type of DLT
			Total	DLT in first course	
1	250	60	3	0	
2	250	80	6	2	ALP increase; AST/ALT increases

Safety

A total of 144 cycles of chemotherapy was administered, with a median of 6 treatment cycles per patient (range 1–19). The major AEs during the entire treatment period are shown in Table 3. The most frequent (≥50 %) AEs were anemia, rash, hyperpigmentation, nausea, anorexia, fatigue, diarrhea, stomatitis, AST elevation, ALT elevation, and hyperbilirubinemia, all of which were clinically manageable. At dose level 2 ($n = 17$), hematologic AEs of grade ≥3 were not observed, and nonhematologic toxicities of grade 3 included stomatitis, increased ALP, increased AST, and increased ALT (6 % each). Nonhematologic AEs of grade 4 were not apparent. Interstitial lung disease was not manifest in any patient, and there were no treatment-related deaths.

Pharmacokinetics

Eight patients (three at dose level 1 and five at dose level 2) in the dose-escalation phase of the study were evaluable for pharmacokinetics. The mean steady-state pharmacokinetic parameters for gefitinib (250 mg daily) administered alone or with S-1 are summarized in Table 4. There were no substantial differences in the mean values of the area under the plasma concentration–time curve over 24 h (AUC_{0-24}) or the maximal concentration (C_{max}) for gefitinib when this drug was administered with or without S-1, suggesting that S-1 at either dose did not affect the trough levels of gefitinib.

Pharmacokinetic analysis was also performed for the plasma concentrations of S-1 components (FT, CDHP, and Oxo) and the FT metabolite 5-FU on the first day of gefitinib and S-1 combination therapy. The increases in the mean values of AUC_{0-8} and C_{max} for FT, 5-FU, and CDHP at dose level 2 compared with those at dose level 1 were consistent with the increase in S-1 dose (Table 5), and the pharmacokinetic parameters obtained for S-1 at dose level 2 administered together with gefitinib in the present study did not appear to differ substantially from those obtained previously for S-1 administered alone at 80 mg/m^2 [4].

Efficacy

All 20 patients were evaluable for antitumor response. Three individuals showed a complete response and seven patients showed a partial response, yielding an overall response rate of 50 %. Five patients had stable disease, giving an overall disease control rate of 75 %. In *EGFR* mutation–positive patients ($n = 9$), seven patients achieved an objective response, whereas none with wild-type *EGFR* ($n = 6$) responded. The median PFS and OS for all treated patients were 10.5 months (95 % confidence interval 2.5–12.9 months) and 21.2 months (95 % confidence interval 13.1–26.0 months), respectively. The median PFS was 12.4 and 3.3 months for the *EGFR* mutation–positive patients ($n = 9$) and the patients with wild-type *EGFR* ($n = 6$), respectively.

Discussion

We have previously shown that combined treatment with S-1 and gefitinib has a synergistic antiproliferative effect on NSCLC cells [7]. On the basis of this finding and additional preclinical data, we undertook the present phase I trial to assess the safety–tolerability, pharmacokinetics, and antitumor efficacy of the combination of gefitinib and S-1 in previously treated patients with advanced adenocarcinoma of the lung. Our study has demonstrated that once-daily gefitinib (250 mg) combined with administration of S-1 (80 mg/m^2) for 14 consecutive days every 21 days has an acceptable tolerability profile in such patients, indicating that full single-agent doses of both drugs can be used in combination. Most toxicities were mild or moderate in extent and were similar in type to those observed in monotherapy studies of gefitinib or S-1 [5, 11]. AEs of grade 3 included stomatitis and etlevation of AST, ALT, and ALP levels. All toxicities of grade 3 were reversible and were manageable with symptomatic treatment and dose reduction or interruption. AEs of grade 4 were not observed. The incidence of AEs during combination therapy with gefitinib and S-1 was not higher than that previously determined for either single-agent therapy.

S-1 is an oral fluorinated pyrimidine formulation that combines FT, CDHP, and Oxo. Oxidation of FT (prodrug of 5-FU) is largely dependent on CYP2A6 [12], and 5-FU showed no inhibitory effect on CYP activity in human liver microsomes [13]. Urinary excretion is the primary elimination pathway for CDHP. Non-CYP enzymes, including xanthine oxidase, contribute to the degradation of Oxo. On the other hand, elimination of gefitinib is dependent largely on CYP3A4 and to a lesser extent on CYP2D6 [14, 15]. Given the differences in metabolism and elimination between gefitinib and S-1, no pharmacokinetic interaction

Table 3 Treatment-related adverse events according to treatment cohort and grade

	Level 1 (n = 3)			Level 2 (n = 17)		
	All grades (%)	Grade 3 (%)	Grade 4 (%)	All grades (%)	Grade 3 (%)	Grade 4 (%)
Hematologic						
Anemia	1 (33)	0	0	12 (71)	0	0
Thrombocytopenia	1 (33)	0	0	4 (24)	0	0
Leukopenia	0	0	0	0	0	0
Neutropenia	1 (33)	1 (33)	0	1 (6)	0	0
Nonhematologic						
Rash	3 (100)	0	0	13 (76)	0	0
Hyperpigmentation	1 (33)			10 (59)		
Vomiting	1 (33)	0	0	2 (12)	0	0
Nausea	2 (67)	0	0	4 (24)	0	0
Anorexia	1 (33)	0	0	11 (65)	0	0
Fatigue	2 (67)	0	0	8 (47)	0	0
Diarrhea	2 (67)	0	0	9 (53)	0	0
Stomatitis	2 (67)	0	0	8 (47)	1 (6)	0
ALP increase	1 (33)	0	0	3 (18)	1 (6)	0
AST increase	2 (67)	0	0	8 (47)	1 (6)	0
ALT increase	2 (67)	0	0	8 (47)	1 (6)	0
Hyperbilirubinemia	2 (67)	0	0	7 (41)	0	0

Table 4 Effect of S-1 on the pharmacokinetics of gefitinib

Parameter	Dose level 1 (n = 3)		Dose level 2 (n = 5)	
	Monotherapy	Combination	Monotherapy	Combination
C_{max} (ng/ml)	516.0 ± 100.5	524.1 ± 96.1	684.8 ± 246.9	741.0 ± 208.3
T_{max} (h)	4.8 ± 2.5	5.0 ± 2.0	4.8 ± 2.5	3.8 ± 1.1
$t_{1/2}$ (h)	20.2 ± 2.4	21.3 ± 6.5	21.5 ± 3.8	29.4 ± 9.3
AUC_{0-24} (ng h/ml)	8,567.2 ± 2,131.0	8,849.3 ± 822.8	12,612.7 ± 4,908.2	12,880.9 ± 4,108.6

Data are mean ± SEM

C_{max} maximal plasma concentration of gefitinib, T_{max} time to achieve C_{max}, $t_{1/2}$ plasma half-life of gefitinib, AUC_{0-24} area under the plasma gefitinib concentration–time curve for 0–24 h

between these two agents would be expected. We evaluated the pharmacokinetics of combination therapy with gefitinib and S-1 in the present study. The C_{max} and AUC values of gefitinib obtained here were similar to those determined in phase I trials in patients with solid malignant tumors who received continuous single-agent treatment with gefitinib [16, 17]. To investigate directly the possible effect of S-1 on the pharmacokinetics of gefitinib, we collected blood samples on day 14 during the run-in period of administration of gefitinib alone as well as on the first day of combination therapy with gefitinib and S-1. The plasma concentration profiles and pharmacokinetic parameters for gefitinib were not altered by coadministration of S-1. The pharmacokinetic parameters obtained for S-1 (80 mg/m^2) during gefitinib dosing did not appear to differ substantially from those previously obtained for S-1 administered as a single agent [4], suggesting that gefitinib affects neither the conversion of FT to 5-FU nor the biological behavior of CDHP or Oxo. Together, these data thus indicate that there was no substantial pharmacokinetic interaction between gefitinib and S-1.

Given that single-agent treatment with EGFR-TKIs is now an established first-line therapeutic option for *EGFR* mutation–positive NSCLC, on the basis of recent phase III trials comparing EGFR-TKIs with platinum-based chemotherapy [18–21], it seems reasonable to test EGFR-TKIs in combination with other chemotherapeutic agents in such patients. The promising safety profile and apparent lack of pharmacokinetic interaction observed for the combination of S-1 and gefitinib in our phase I study suggest that this drug combination is a new treatment option for *EGFR* mutation–positive patients with advanced NSCLC.

Table 5 Pharmacokinetic parameters for S-1 components and 5-FU at the two dose levels

Parameter	Dose level 1 ($n = 3$)				Dose level 2 ($n = 5$)			
	FT	5-FU	CDHP	Oxo	FT	5-FU	CDHP	Oxo
C_{max} (ng/ml)	1,445.0 ± 228.0	101.9 ± 42.9	130.7 ± 72.3	54.5 ± 49.8	1,798.0 ± 138.0	182.1 ± 63.8	251.8 ± 56.5	44.2 ± 21.5
T_{max} (h)	2.0 ± 1.0	4.0 ± 1.0	3.0 ± 0.0	3.0 ± 0.0	2.0 ± 1.0	3.0 ± 0.0	3.0 ± 1.0	3.0 ± 0.0
$t_{1/2}$ (h)	6.13 ± 0.96	1.73 ± 0.30	2.64 ± 0.54	2.95 ± 0.97	6.55 ± 1.37	1.56 ± 0.34	2.41 ± 0.40	2.57 ± 0.96
AUC_{0-8} (ng h/ml)	7,446.0 ± 1,546.0	454.0 ± 193.4	532.1 ± 242.2	208.9 ± 145.9	9,752.0 ± 956.0	794.6 ± 280.1	1,000.6 ± 246.9	190.7 ± 85.0

Data are mean ± SEM

A further clinical concern is that *EGFR* mutation–positive patients who initially respond to EGFR-TKIs eventually develop resistance to these agents. At present, no drug that is able to overcome such acquired resistance is available in clinical practice. We have previously shown that the combination of gefitinib and S-1 has a synergistic antiproliferative effect on EGFR mutation–positive NSCLC cells that have developed resistance to EGFR-TKIs [8]. The addition of S-1 to gefitinib may thus prove effective for the treatment of *EGFR* mutation–positive patients with acquired resistance to EGFR-TKIs.

In conclusion, combination therapy with gefitinib (250 mg/day) and S-1 (80 mg/m^2 for 14 days every 21 days) was well tolerated in previously treated patients with advanced pulmonary adenocarcinoma. Further studies are thus warranted to confirm the efficacy and safety of combination therapy with S-1 and gefitinib in comparison with gefitinib monotherapy.

Conflict of interest The authors declare no conflict of interest.

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Histology and Smoking Status Predict Survival of Patients with Advanced Non–Small-Cell Lung Cancer

Results of West Japan Oncology Group (WJOG) Study 3906L

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Introduction: Smoking status is one of the prognostic factors in advanced non–small-cell lung cancer (NSCLC). Currently, adenocarcinoma (Ad) histology is considered a predictive factor in advanced NSCLC. We investigated the correlation between histology or smoking status and survival of NSCLC patients receiving chemotherapy.

Methods: We retrospectively reviewed clinical data from stage IIIB or IV NSCLC patients who started first-line chemotherapy at affiliated institutions of West Japan Oncology Group from 2004 to 2005. We also collected information on pack-years of cigarette smoking and years since cessation. Overall survival was compared using log-rank test, and Cox regression analysis was used to identify independent prognostic factors.

Results: In total, 2542 consecutive patients were enrolled at 40 institutions. Of those, 71 were excluded because of unknown smoking history. The median overall survival of nonsmoking Ad patients (593 days) was longer than that of smoking Ad, nonsmoking non-Ad, and smoking non-Ad patients (384, 374, and 319 days, respectively; $p < 0.001$). In Cox regression with sex, age, stage, performance, and treatment as covariates, we found significant interaction ($p = 0.039$) between histology (Ad/non-Ad) and smoking status (smoker/nonsmoker); smoking conferred a hazard ratio of 1.34 (95% confidence interval, 1.15–1.55) in Ad, but only 0.99 (0.75–1.31) in non-Ad. Higher pack-years and shorter period since cessation were significantly associated with poorer survival in Ad ($p < 0.001$), but not in non-Ad ($p \geq 0.434$).

Conclusion: Ad histology is associated with better prognosis, and only smoking status had a prognostic impact in Ad.

Key Words: Non–small-cell lung cancer, Histology, Adenocarcinoma, Smoking status.

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This study is registered with University Hospital Medical Information Network-Clinical Trial Registry (UMIN-CTR) (http://www.umin.ac.jp/ctr/index.htm umin.ac.jp/ctr; identification number UMIN000001263).

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Lung cancer is the leading cause of cancer-related mortality in Japan, and the rest of the world, with more than one million people dying from it each year. Non–small-cell lung cancer (NSCLC), which accounts for nearly 80% of all lung cancers, comprises several histological types, including adenocarcinoma (Ad), squamous cell carcinoma (Sq), and large-cell carcinoma (La). NSCLC had been treated as a single disease because of similar therapeutic effects of conventional chemotherapeutic agents. In the last few decades, however, treatment with new drugs, such as epidermal

growth factor receptor tyrosine kinase inhibitors (EGFR-TKIs), bevacizumab, and pemetrexed revealed that tumor histology has profound impact on the benefits of a variety of chemotherapy or targeted-therapy regimens for advanced NSCLC.[1-4] Thus, histology came to be considered a predictive factor for the effectiveness of specific chemotherapy in patients with advanced NSCLC. However, there is no previous report on histology as a prognostic factor, that is, a variable determining survival irrespective of the chemotherapy regimen administered.

Previous studies showed that cigarette smoking is an independent prognostic factor in patients with NSCLC,[2,5-7] but a dose–response relationship between the quantity of smoking and survival has not been established. Although Yelena et al.[6] noted that patients who had smoked up to 15 pack-years had a longer survival than those with more than a 15 pack-year history, other cutoff points for the amount of cigarette smoking have not been considered. In addition, the relationship between smoking and survival was not investigated with respect to differences in NSCLC histological subtypes, and the studies that did evaluate survival in Sq versus non-Sq patients did not reach a firm conclusion.[7,8] However, Kawaguchi et al.[8] showed that Ad had better prognosis than Sq in never-smokers, but not in ever-smokers, suggesting that the prognostic impact of cigarette smoking may differ among histologic subtypes in NSCLC.

We hypothesized that Ad histology and lower smoking status would result in better overall survival (OS) in advanced NSCLC. To test this hypothesis, we investigated the impact and possible interaction of histology and smoking status on survival of advanced NSCLC patients receiving chemotherapy in the clinic.

PATIENTS AND METHODS

Study Patients

We sent case report forms to 40 affiliated institutions of West Japan Oncology Group, and requested them to provide demographic and clinical data from medical records for all patients with stage IIIB or IV NSCLC, who started first-line systemic chemotherapy between January 1, 2004 and December 31, 2005. Patients who had a relapse after surgery or radiotherapy were excluded. The case report forms were submitted by the participating institutions during the period from September 2008 to January 2009. This study was approved by the institutional review board of each participating institution.

Demographic and Clinical Variables

We obtained the following baseline demographic and clinical information from the case report forms: age, sex, histology, disease stage, Eastern Cooperative Oncology Group performance status (PS), smoking status, type of first-line chemotherapy, number of treatment regimens, and the year in which first-line chemotherapy was started. Disease stage was determined according to the tumor, node, metastasis system.[9] Staging classification was performed by physical examination, chest–abdominal computed tomography, brain magnetic resonance imaging, bone scan, and positron emission tomography if necessary. Patients were categorized into nonsmokers and smokers according to smoking status. Nonsmokers were defined as those who had smoked less than 100 cigarettes. Among smokers, exsmokers were defined as those who had quit smoking 1 year or more before diagnosis, and current smokers as those who continued their smoking habit at diagnosis. Pack-years of smoking were calculated by multiplying the number of packs (20 cigarettes in one pack) smoked per day by the number of years smoked, and categorized as less than 10, 10 to 19, 20 to 29, 30 to 39, 40 to 49, 50 to 59, and 60 or more. Years of smoking cessation were categorized as 1 to 4, 5 to 9, 10 to 14, 15 to 19, and 20 or more. Type of first-line chemotherapy was categorized into platinum-based combination, nonplatinum combination, and single-agent chemotherapy. Because the only approved EGFR-TKI for the treatment of inoperable or recurrent NSCLC in Japan before October 2007 was gefitinib, we collected information on gefitinib usage during the observation period and noted the starting day of gefitinib treatment. OS was calculated from the start of first-line chemotherapy to the date of death. Patients still alive were censored as of the last known follow-up.

TABLE 1. Patient Characteristics

Parameter	Ad (n = 1731)	Non-Ad (n = 740)	p
Men/women	1056/675	641/99	<0.001
Smoking status			<0.001
Nonsmoker	659	79	
Exsmoker	300	165	
Current smoker	772	496	
Stage IIIB/IV	444/1287	271/469	<0.001
PS			0.002
0	546	206	
1	873	402	
2	191	96	
3	90	25	
4	31	11	
Histology			—
Sq	—	516	
La	—	71	
Others	—	153	
Chemotherapy			0.181
Single-agent	354	137	
P doublet	1306	571	
Non-P doublet	71	32	
Regimen			<0.001
1	536	285	
2	445	201	
3	322	115	
≥4	428	139	
Gefitinib Y/N	959/772	146/594	<0.001

Ad, adenocarcinoma; PS, performance status; Sq, squamous cell; La, large cell; P, platinum; Y, yes; N, no.

Statistical Analysis

Demographic and clinical variables were compared among groups according to lung cancer histology, using the χ^2 test. The primary endpoint of this study was OS. Survival curves were calculated by the Kaplan–Meier method and compared using the log-rank test. Prognostic importance of histology and smoking status were analyzed using the Cox regression analysis adjusted for sex, age, disease stage, PS, type of first-line chemotherapy, and the year in which first-line chemotherapy was started. For detection of possible interaction between histology and smoking status, the terms of interaction of the two variables were evaluated by the likelihood ratio test. Because gefitinib was the preferred choice in patients with Ad, another Cox regression analysis was performed, in which patients were censored at the start of gefitinib administration, and the results were compared with the original Cox analysis. Significance level was set at a p value of 0.05. Statistical analyses were performed with SAS version 9.2 software (SAS Institute, Cary, NC).

RESULTS

Between January 1, 2004 and December 31, 2005, 2542 consecutively treated patients were enrolled at 40 institutions. Of these, 71 were excluded because of unknown smoking history. The characteristics of the study population, categorized into Ad and non-Ad, are listed in Table 1. There were 1731 Ad and 740 non-Ad patients (29.9% and 70.1%, respectively). Among them, we confirmed 1346 and 599 deaths in Ad and non-Ad patients, respectively. There were significantly more women (39.0% in Ad versus 13.4% in non-Ad) and nonsmokers (38.1% in Ad versus 10.7% in non-Ad) in the Ad group than in the non-Ad group. Patients who received single-agent chemotherapy accounted for approximately 20% of the study population. Compared with combination regimens, single-agent chemotherapy was associated with old age (63.6 years for combination regimens versus 71.1 years for single-agent chemotherapy), high proportions of female patients (29.3% versus 40.0%), nonsmokers (27.8% versus 34.0%), stage IV (69.4% versus 78.3%), and PS 0 to 1 (60.9% versus 87.1%). The proportion of Ad histology was not significantly different between single-agent and combination regimens (72.1% and 69.5%, respectively). The OS was 464 days in Ad compared with 326 days in non-Ad ($p < 0.001$; Fig. 1*A*). Between Ad and non-Ad, which was divided into Sq and La, Ad had significantly better survival than the other two histological groups (Sq, 341 days; La, 254 days; $p < 0.0001$; Fig. 1*B*). With regard

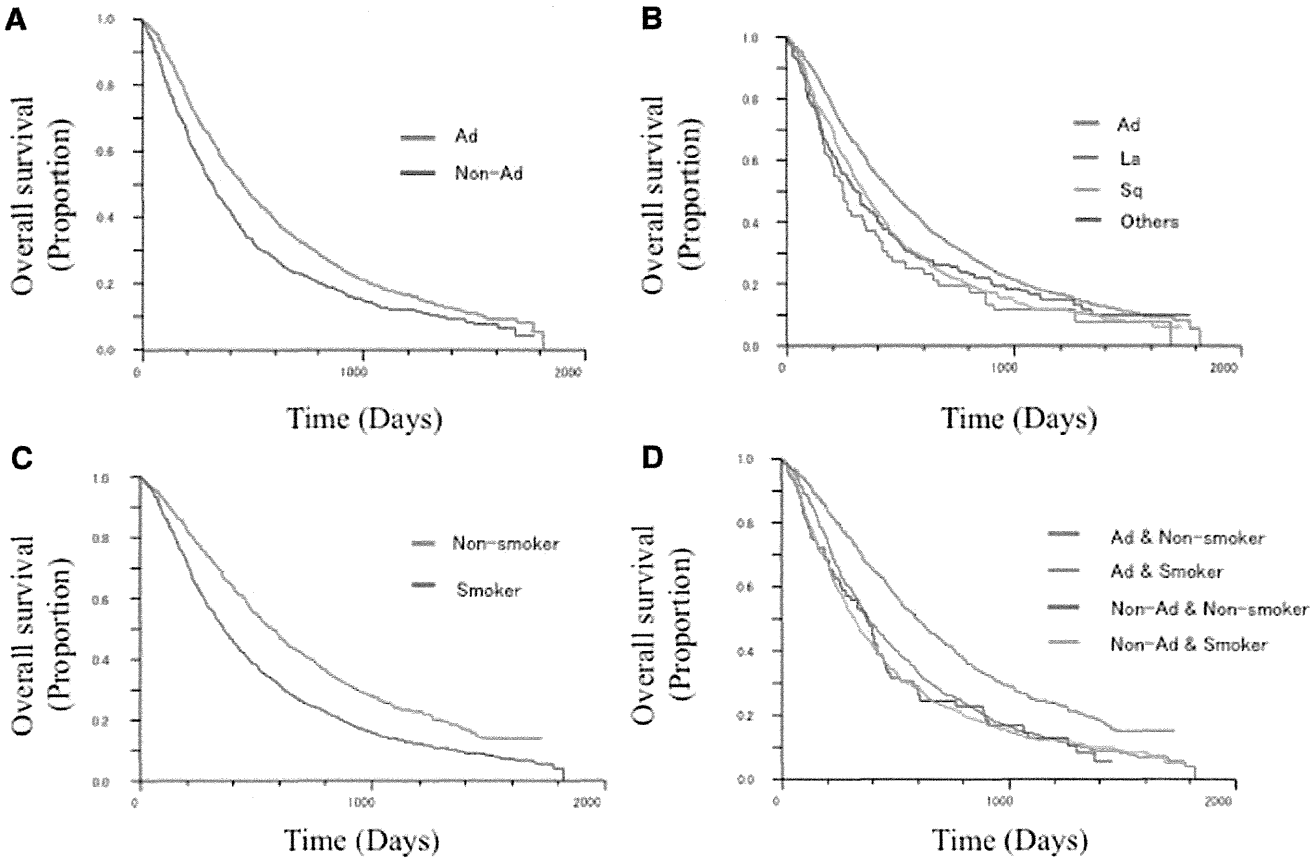

FIGURE 1. Kaplan–Meier plots of overall survival for patients classified according to histology type as (*A*) Ad and Non-Ad; histologic subtype as (*B*) Ad, La, Sq, and others; smoking status as (*C*) smokers and nonsmokers; and combination of smoking status and histology as (*D*) Ad and nonsmoker, Ad and smoker, Non-Ad and nonsmoker, and Non-Ad and smoker. Ad, adenocarcinoma; La, large cell; Sq, squamous cell.

TABLE 2. Survival Analysis by Cox Proportional Hazards Model ($n = 2471$)

Parameter	HR	95% CI	p
Sex			
Women	1		
Men	1.342	1.168–1.541	<0.001
Age yrs	1.007	1.002–1.012	0.005
Smoking status			
Nonsmoker	1		
Exsmoker	1.178	0.997–1.391	0.054
Current smoker	1.335	1.155–1.543	<0.001
Clinical stage			
Stage IIIB	1		
Stage IV	1.505	1.358–1.669	<0.001
PS			
0	1		
1	1.609	1.446–1.790	<0.001
2	2.229	1.910–2.601	<0.001
3	3.048	2.455–3.785	<0.001
4	5.487	3.864–7.790	<0.001
Histology			
Ad	1		
Sq	1.143	1.015–1.286	0.028
La	1.542	1.182–2.011	0.001
Others	1.397	1.159–1.683	<0.001
Chemotherapy			
Single-agent	1		
Non-P doublet	0.842	0.657–1.080	0.175
P doublet	0.793	0.699–0.899	<0.001

HR, hazard ratio, CI, confidence interval; PS, performance status; Ad, adenocarcinoma; Sq, squamous cell; La, large cell; P, platinum.

to smoking status, nonsmokers (568 days) had significantly longer survival than smokers (358 days; $p < 0.0001$; Fig. 1*C*). In a combined analysis of smoking status and histology, the median OS of Ad in nonsmokers was longer than that of Ad in smokers, non-Ad in nonsmokers, and non-Ad in smokers (593, 384, 374, and 319 days, respectively; $p < 0.001$; Fig. 1*D*). In Cox regression analysis, sex, age, smoking status, disease stage, PS, histology, and chemotherapy showed a statistically significant prognostic impact on survival (Table 2). When the interaction between histology (Ad/non-Ad) and smoking status (smoker/nonsmoker) was included in the Cox model, significant interaction was observed ($p = 0.039$); smoking conferred a hazard ratio (HR) of 1.34 (95% confidence interval [CI], 1.15–1.55) in Ad, in contrast to 0.99 (0.75–1.31) in non-Ad. In detailed analyses that excluded the 104 patients (current smokers, 89; unknown, 15) with unknown amount of cigarette smoking, shorter period since cessation showed a significant trend for poorer survival in the whole population ($p < 0.001$). This trend was also observed in Ad ($p < 0.001$; Table 3), but not in non-Ad ($p \geq 0.434$; Table 3). When non-Ad patients were divided into Sq and La or others, the trend p was 0.534 in Sq and 0.165 in La or others. The prognosis became significantly worse with higher pack-years of cigarette smoking in the whole population and Ad ($p < 0.001$; Table 3), but no significance was not achieved for the non-Ad group ($p = 0.519$; Table 3). When non-Ad patients were divided into Sq and La or others, the trend p was 0.798 in Sq and 0.380 in La or others. The prognostic impact of histology and smoking status remained significant in the Cox regression analysis, in which patients were censored at the start of gefitinib administration; positive smoking history, Sq histology, and La or other histology conferred an HR of 1.51 (95% CI, 1.21–1.88), 1.22 (95% CI, 1.06–1.41), and 1.59 (95% CI, 1.32–1.93), respectively. The negative prognostic impact of shorter period since cessation and pack-years of cigarette smoking was also essentially unchanged ($p < 0.001$ in both).

DISCUSSION

The consensus report of prognostic factors in NSCLC at the 1990 International Association for the Study of Lung Cancer Workshop showed that histology was not a prognostic factor for advanced NSCLC.[10] Our study is the first report to reveal that histology is a significant prognostic factor for advanced NSCLC. Importantly, we showed that Ad patients have the longest survival of all three histological groups (Ad, Sq, and La). Ad is the most common histological subtype of lung cancer in nonsmokers,[11] who have been reported to have a better prognosis than smokers.[12–14]

Smoking has been described as a prognostic factor in lung cancer. Although multiple studies have demonstrated the negative effects of smoking in patients with NSCLC, most included a heterogeneous population comprising patients with all stages and types of lung cancer.[5] In contrast, our study cohort consisted exclusively of patients with advanced NSCLC treated with first-line chemotherapy. We showed that smoking status is an independent prognostic factor for survival in those patients. Similar data have been shown in former studies.[2,5] However, those reports did not show whether smoking conferred any survival impact for advanced NSCLC irrespective of histological subtypes. In our study, only Ad histology had significant interaction with smoking status or smoking index and prognosis. A higher level of smoking was related to shorter survival in Ad patients, whereas smoking level and survival were not associated in non-Ad patients. Although the proportion of non-Ad patients was 29.9% of the total, the observed number of deaths in this study yielded a statistical power of more than 80% for detecting an HR of 1.5 at the 5% significance level in both Ad and non-Ad patients. Others have found that Ad histology is a significant prognostic factor in separate multivariate analysis for never-smokers in advanced NSCLC.[8] Yelena et al.[6] showed that high cigarette smoking, as measured in pack-years, is associated with decreased survival after diagnosis of stage IIIB/IV NSCLC. However, the patients of that study received a wide variety of therapies, raising the possibility that the outcomes might have been the result of distinct therapeutic responses. Although we only assessed the prognostic value of smoking status at diagnosis, assessment of smoking status at a later point, that is, at the time of treatment, would also have been of interest to determine whether cessation at the time of diagnosis leads to improved survival.

TABLE 3. Hazard Ratios According to Quantitative Aspects of Smoking

	Ad			Non-Ad		
	HR	95% CI	*p*	HR	95% CI	*p*
Years after cessation	(*n* = 1731)			(*n* = 740)		
Current	1.492	1.271–1.750	<0.001	1.204	0.849–1.707	0.297
Exsmoker 1–4 yr	1.438	1.114–1.857	0.005	1.101	0.733–1.653	0.643
Exsmoker 5–9 yr	1.549	1.101–2.180	0.012	1.228	0.700–2.155	0.474
Exsmoker 10–14 yr	1.127	0.783–1.621	0.520	1.235	0.680–2.245	0.488
Exsmoker 15–19 yr	1.199	0.761–1.890	0.433	1.410	0.712–2.794	0.325
Exsmoker ≥20 yr	0.873	0.834–1.203	0.407	1.103	0.662–1.837	0.706
Trend *p*	<0.001			0.434		
Pack-yr	(*n* = 1665)			(*n* = 702)		
<10	1.267	0.899–1.785	0.176	1.196	0.535–2.672	0.662
10–19	1.118	0.801–1.561	0.513	0.963	0.512–1.812	0.908
20–29	1.346	1.048–1.729	0.020	1.368	0.887–2.109	0.157
30–39	1.345	1.071–1.689	0.011	0.954	0.624–1.458	0.827
40–49	1.370	1.096–1.712	0.006	1.128	0.763–1.669	0.546
50–59	1.483	1.164–1.890	0.001	1.238	0.828–1.851	0.298
≥60	1.595	1.312–1.939	<0.001	1.135	0.791–1.628	0.491
Trend *p*	<0.001			0.519		

[a] Nonsmokers were set as the reference category.
Ad, adenocarcinoma; HR, hazard ratio; CI, confidence interval.

In agreement with the findings of another study,[15] we also found that a large proportion of Ad patients were nonsmoking. The prognostic difference between Ad in never-smokers and smokers may suggest that both are different disease entities. Of note, tumor-mutational frequencies and spectra suggest differences between smokers and nonsmokers.[16,17] However, significant differences in the frequency of somatic mutations in oncogenes such as *EGFR* and *KRAS* have been observed between smoking and nonsmoking lung cancer patients.[11] *EGFR* mutations, clinical predictors of EGFR-TKI therapeutic benefits, are more frequently found in nonsmoking Ad patients.[11] In another study, *EGFR* mutations were identified in nonsmokers (51%), former smokers (19%), and current smokers (4%).[18] Moreover, the incidence of *EGFR* mutations decreased with increasing number of pack-years of cigarette smoking.[18] However, *KRAS* mutations, predicting poor survival and resistance to EGFR-TKI, are more frequently found in smoking Ad patients. Interestingly, *EGFR* and *KRAS* mutations are mutually exclusive.[11]

Currently, therapeutic options other than EGFR-TKIs (e.g., bevacizumab and pemetrexed) are available in Japan. Still, NSCLC subtypes have been showing variable response rates and adverse events.[2,4,19,20] Non-Sq histology, especially Ad, is currently the NSCLC subtype with broader and more efficacious treatment options. At the time of this study, however, the only approved therapeutic agent for NSCLC in Japan was gefitinib. Unfortunately, we did not investigate *EGFR* mutation status. However, genetic background could possibly predict response to gefitinib. Along with its retrospective nature, this was a limitation of our study. However, we found that the treatment choice was made on the basis of clinical background, and we were unable to conclude whether or not gefitinib contributed to better survival under unknown *EGFR* mutation status. Hence, we suggest that decision-making based on clinical information alone is inappropriate. Both the V15-32 study[21] and the Iressa Survival Evaluation in Lung Cancer (ISEL) study[22], support our observations. Furthermore, the IRESSA Pan-Asia Study (IPASS) study,[23] conducted under the hypothesis that EGFR-TKI would be effective in clinically selected patients, confirmed the strong predictive value of *EGFR* mutations for the response of Ad to gefitinib.

This retrospective study has a few other limitations as well. First, information on smoking was not obtained from the interview or the self-administered questionnaire. Smoking data can be inaccurate, particularly when collected retrospectively. Second, we did not collect data on the procedures for histological diagnosis. The basis for pathological diagnosis is important because cytological assessment alone may lead to underdiagnosis of specific histologic types.

In conclusion, this survey demonstrated that Ad histology is associated with better prognosis, and that smoking status has a prognostic impact only in patients with Ad.

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Interstitial Lung Disease Associated with Gefitinib in Japanese Patients with *EGFR*-mutated Non-small-cell Lung Cancer: Combined Analysis of Two Phase III Trials (NEJ 002 and WJTOG 3405)

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Objective: Interstitial lung disease associated with gefitinib is a critical adverse reaction. When geftinib was administered to *EGFR*-unknown patients, the interstitial lung disease incidence rate was approximately 3–4% in Japan, and usually occurs during the first 4 weeks of treatment. However, it has not been fully investigated in *EGFR*-mutated patients.
Methods: We collected clinical records of participants of two Phase III trials (WJTOG 3405 and NEJ 002), which compared gefitinib with platinum doublet chemotherapy. All patients were *EGFR* mutated, chemo-naïve and had good performance status.
Results: A total of 402 patients were enrolled in this study. In the gefitinib arm, 10 (5.0%) of 201 patients developed interstitial lung disease, of whom five (2.5%) were Grade 3 or greater, with two deaths (1.0%). In contrast, only one patient developed interstitial lung disease (Grade 1) in the chemotherapy arm. With regard to gefitinib, smoking history was significantly associated with developing interstitial lung disease (odds ratio 0.18; 95% confidence interval: 0.05–0.74; $P = 0.01$). The cumulative incidence rate of interstitial lung disease was similar in the 0–4, 5–8 and 9–12 week time periods. However, between smokers and never-smokers, cumulative incidence rates in the first 4 weeks were significantly different (4.7% versus 0%, $P = 0.03$). Three of 10 patients developed interstitial lung disease after 8 weeks of gefitinib administration (days 135, 171 and 190, respectively).
Conclusions: Among *EGFR*-mutated patients, the incidence of interstitial lung disease associated with gefitinib was not different from that in previous reports. Smoking history was associated with developing interstitial lung disease, and smokers had a higher incidence rate of interstitial lung disease in the first 4 weeks.

Key words: epidermal growth factor receptor mutation – gefitinib – epidermal growth factor receptor-tyrosine kinase inhibitor – interstitial lung disease – Japanese

INTRODUCTION

The recent introduction of targeted agents has dramatically changed the treatment of non-small-cell lung cancer (NSCLC). Epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor (TKI) is a prototype of such therapy which targets NSCLC harboring the *EGFR* mutation (1,2). EGFR-TKIs have demonstrated a higher response rate and longer progression-free survival than platinum doublet chemotherapy (3–6). Common adverse events associated with EGFR-TKIs include skin rash, diarrhea and hepatotoxicity. Interstitial lung disease (ILD) is a rare but potentially fatal adverse event (7). The incidence of ILD has been reported to be higher in Japanese than in Caucasians. Two large, multi-institutional studies in Japan (8–10) reported that its incidence is 3.5–4.0%, compared with just 0.3% in the USA (11). They also suggested that male gender, history of smoking, poor performance status, pre-existing lung disorder and prior history of chemotherapy were predictive risk factors (8–10).

Today, clinical guidelines recommend that administration of EGFR-TKIs should be limited to *EGFR*-mutated patients, reflecting the high efficacy of this drug in this patient population (12). Since it is known that *EGFR* mutation is relatively rare in males or smokers, which are known risk factors of ILD, ILD incidence might be lower in patients with *EGFR* mutation. However, a detailed investigation of ILD associated with EGFR-TKIs among *EGFR*-mutated patients has not been done. Therefore, we conducted a combined analysis of two Phase III trials that compared gefitinib with platinum doublet chemotherapy in Japanese NSCLC patients with *EGFR* mutation.

PATIENTS AND METHODS

Patient Selection and Treatment Methods

We collected the clinical records of participants of two Phase III trials (WJTOG 3405 (3) and NEJ 002 (4)). These trials compared gefitinib with platinum doublet chemotherapy in Japanese NSCLC patients with *EGFR* mutation. *EGFR* mutation was screened by PCR-based methods as previously described (13,14). All of the participants were required to be chemo-naïve, with Eastern Cooperative Oncology Group performance status (ECOG PS) of 0–1 and aged between 20 and 75 years, with adequate organ function. Patients with active infectious disease or severe heart disease were excluded. All patients were confirmed not to have pulmonary fibrosis by chest computed tomography (CT) within 1 month prior to registration. Both studies were approved by the institutional review board at each participating site.

Eligible patients were randomly assigned to receive either gefitinib (250 mg daily) or standard chemotherapy. The latter consisted of paclitaxel 200 mg/m^2 plus carboplatin (area under the curve of six) in NEJ 002 or docetaxel 60 mg/m^2 plus cisplatin 80 mg/m^2 in WJOG 3405, every 3 weeks. All participants who had received at least one dose of a study drug were included in the safety analysis.

Baseline data were collected for each patient, including information on sex, age, history of smoking, ECOG PS, tumor histology, clinical stage and type of *EGFR* mutation.

Evaluation of ILD and Statistical Analysis

All patients were assessed by chest CT for their response to treatment every 2 months. The diagnosis of ILD was based on clinical manifestations (worsening dry cough or dyspnea within days to weeks), accompanied by interstitial pulmonary infiltrates on a chest X-ray and a chest CT (15). Close investigation, such as blood and bacterial examination, was required in the protocols to exclude other ILDs. Bronchoalveolar lavage was also recommended, if possible. ILD was assessed according to the National Cancer Institute

Table 1. Baseline characteristics of the patients in the gefitinib arm

	Total ($n = 201$)	Non-ILD ($n = 191$)	ILD ($n = 10$)	*P* value
Age (years)				
Mean	64	64	63	0.67
Range	34–75	34–75	56–75	
Sex (no.)				
Male	71	65	6	0.17
Female	130	126	4	
Smoking status (no.)				
Never	137	134	3	0.01
Previous/current	64	57	7	
ECOG performance status (no.)				0.35
0	111	107	4	(PS 0 versus 1)
1	89	83	6	
2	1	1	0	
Histology (no.)				
Ad	187	180	7	1.0
Other	14	14	0	
Clinical stage (no.)				
IIIB	25	25	0	0.52
IV	129	122	7	
Post-operative relapse	47	44	3	
Type of *EGFR* mutation				
Exon 19 del	108	104	4	0.42
L858R	85	80	5	
Other	8	7	1	

ILD, interstitial lung disease; ECOG, Eastern Cooperative Oncology Group; *EGFR*, epidermal growth factor receptor.

Common Terminology Criteria (NCI-CTC, version 3.0). All events were assessed by investigators at first; then severe cases were confirmed by independent committees based on medical, pathological and radiological findings.

Differences between covariates in patients with or without ILD were analyzed using Fisher's exact tests or Pearson's tests. The Kaplan–Meier method was used to estimate the cumulative incidence rate of ILD, and differences according to the smoking status were analyzed by the log-rank test. All the analyses were performed using JMP version 7 (SAS Institute Inc., USA).

RESULTS

In WJOG 3405, 177 patients were randomized and 175 were included in the safety analysis. In NEJ 002, 230 patients were randomized and 227 were included in the safety analysis. In our study a total of 402 patients were enrolled, half of them in the gefitinib arm.

Baseline characteristics of the patients were well balanced between the treatment groups. As previously reported (3,4), about two-thirds of patients were female, the median age was 64 years, 65% were never-smokers, 55% had an ECOG PS of 0 and 95% had adenocarcinoma.

At the time of data cut-off, the median duration of gefitinib treatment was 165 days (WJTOG 3405) and 308 days (NEJ 002); the median number of chemotherapy cycles was four. In the gefitinib arm, 10 (5.0%) of 201 patients developed ILD, of whom five (2.5%) were Grade 3 or greater, with two deaths (1.0%). In contrast, only one patient developed ILD (Grade 1) in the chemotherapy arm.

The background and clinical course of the patients in the gefitinib arm are summarized in Tables 1 and 2. The clinical background of patients who developed ILD and those who did not showed no difference other than smoking status. Univariate analysis showed that smoking history was significantly associated with developing ILD (odds ratio 0.18; 95% confidence interval (CI): 0.05–0.74; $P = 0.01$). This accounted for 10.9% (95% CI: 5.4–20.9%) of the incidence rate of ILD among smokers, versus 2.2% (95% CI: 0.8–6.3%) among never-smokers.

Figure 1 shows a Kaplan–Meier curve of the cumulative incidence rate of ILD. Among the overall population, the cumulative incidence rate in the first 4 weeks, 5th–8th weeks and 9th–12th weeks was 1.5% (95% CI: 0.5–4.3%), 1.5% (95% CI: 0.5–4.4%) and 0.5% (95% CI: 0.1–2.9%), respectively. Smoking status was associated with the timing of the onset of ILD . Between smokers and never-smokers, the cumulative incidence rate of ILD in the first 4 weeks was significantly different (4.7 versus 0%, $P = 0.03$), whereas that in the other periods (5th–8th weeks and 9th–12th weeks) was similar (Fig. 1). Three of 10 patients developed ILD after 8 weeks of gefitinib administration (days 135, 171 and 190, respectively).

Most of the patients who developed severe ILD (Gr $\geq$ 3) were given steroid therapy. One patient was treated with an immunosuppressive agent (cyclosporine). Non-invasive positive pressure ventilation was used in one patient (No. 10) but unfortunately this patient died.

DISCUSSION

Three large studies of ILD associated with EGFR-TKI have been conducted in Japan (Table 3). Ando et al. (8) performed a retrospective study including 1976 NSCLC patients treated with gefitinib and found an incidence rate of 3.5% and mortality rate of 1.6%. In a prospective cohort and nested-case control study by Kudoh et al. (9), cumulative incidence rates during 12 weeks of treatment were 4.0%. They also mentioned that the risk of developing ILD was higher

Table 2. Clinical characteristics of 10 patients who developed ILD in the gefitinib arm

No.	Age	Sex	Smoking index (BI)	PS	Stage	Site of *EGFR* mutation	Onset day from EGFR-TKI	ILD (CTCAE grade)	Outcome
1	69	M	800	0	r	Exon 19	48	1	Improved
2	57	F	0	1	4	Exon 19	70	1	Improved
3	60	M	860	1	4	Exon 21	15	1	Improved
4	56	F	370	1	4	Exon 19	14	1	Improved
5	71	F	0	1	4	Exon 21	171	2	Improved
6	57	M	740	0	r	Exon 19	25	3	Improved
7	68	M	1075	0	4	Exon 21	190	3	Improved
8	75	M	525	1	4	Exon 21	53	3	Improved
9	65	M	1320	0	r	Exon 19	135	5	Died
10	60	F	0	1	4	Exon 21	32	5	Died

BI, Brinkman Index; PS, Eastern Cooperative Oncology Group performance status; EGFR-TKI, EGFR-tyrosine kinase inhibitor; CTCAE, Common Terminology Criteria for Adverse Events; M, male; F, female.

with gefitinib than with chemotherapy (the odds ratio was 3.2). With regard to erlotinib, Nakagawa et al. (10) conducted a post-marketing survey in Japan and reported that 158 of 3488 patients were confirmed to have ILD (any grade, 4.5%), with a mortality rate of 1.6%. These studies suggested that male gender, smoking history, poor PS, pre-existing lung disorder and prior history of chemotherapy were risk factors of ILD. However, none of the three studies mentioned *EGFR* mutation status.

To our knowledge, ours is the first study to describe the clinical characteristics of ILD associated with gefitinib limited to *EGFR*-mutated patients. Similar to Kudoh's report, ILD was relatively more common in the gefitinib arm than in the chemotherapy arm. The incidence rate of ILD associated with gefitinib was as high as 5% with a mortality rate of 2.5%, even though our analysis contained a high proportion of patients from low-risk groups (female, non-smokers with good PS).

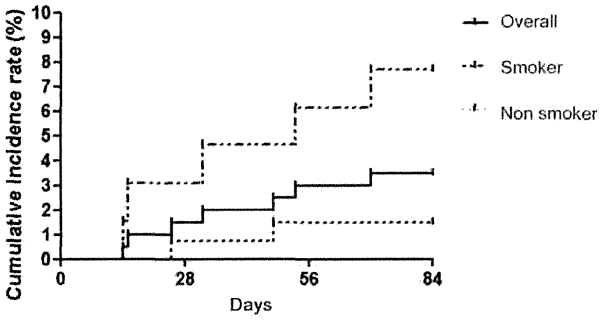

Figure 1. Cumulative incidence rate of interstitial lung disease associated with gefitinib. Kaplan–Meier-estimated cumulative incidence rate of interstitial lung disease in patients who were allocated to the gefitinib arm in WJTOG 3405 and NEJ 002 trial (overall population ($n = 201$), bold line; smoker ($n = 64$), dashed line; non-smoker ($n = 137$), dotted line).

Similarly to the previous studies, our analysis showed that smoking history was highly associated with developing ILD associated with gefitinib (odds ratio 0.18). Smoking induces airway epithelial damage, and lung injury could be prolonged and worsened by gefitinib in a preclinical model (16). Most of the other risk factors were excluded at the time of registration, because enrolled patients were required to be chemo-naïve, with a PS of 0–1, and confirmed not to have pulmonary fibrosis. Therefore, we should pay more attention to smoking status even if the patient has *EGFR* mutation. In terms of the timing of the onset of ILD, smoking history seemed to be an important factor. Between smokers and never-smokers, the cumulative incidence rate of ILD in the first 4 weeks was significantly different (4.7 versus 0%, $P = 0.03$). Previous studies stated that ILD occurred most commonly in the first 4 weeks (median: 23–31 days) and 60% of participants were smokers. So, despite the small subset analysis in the present study, the higher incidence rate observed in the first 4 weeks among smokers is noteworthy.

Another point is that three of 10 patients developed ILD after several months of gefitinib treatment. With erlotinib, it was reported that ILD occurred at the rate of 0.11 per 100 patient-weeks after 8 weeks of treatment. It is not clear whether the mechanism of ILD varies over time from its onset; further investigation on late-onset ILD is needed.

Our analysis has several limitations. First, this was an investigator-dependent analysis. Most of the ILD cases were diagnosed by clinical manifestations and a chest CT. Bronchoalveolar lavage was recommended in the protocols, but actually done in only one case. As acute exacerbation of ILD after bronchoscopy has been reported (15), this may be acceptable. In our analysis, all patients were assessed by chest CT every 2 months, and severe cases were confirmed by independent, multidisciplinary committees. Secondly, this analysis was done with a small sample size due to the population and rarity of incidence.

Table 3. ILD associated with EGFR-TKI in Japanese patients: pivotal studies and ours

	Ando et al. (8)	Kudoh et al. (9)	Nakagawa et al. (10)	Present data
Study design	Retrospective	Prospective	Retrospective	Retrospective
No. of patients	1976	1482	3488	201
Type of EGFR-TKI	Gefitinib	Gefitinib	Erlotinib	Gefitinib
Patient selection by *EGFR* mutation status	No	No	No	Yes
ILD (any Grade; %)	70 (3.5)	59 (4.0)	158 (4.5)	10 (5.0)
ILD (Grade 5; %)	31 (1.6)	25 (1.7)	55 (1.6)	2 (1.0)
Risk factors of ILD	Smoking Pre-existing lung disorder Male	Smoking Pre-existing lung disorder Poor PS Elderly Cardiac disease	Smoking Pre-existing lung disorder Poor PS Lung infection	Smoking

In conclusion, the incidence of ILD associated with gefitinib among *EGFR*-mutated patients was not different from that in previous reports. Smoking history was highly associated with developing ILD. In addition, a substantial number of patients developed ILD after several months of gefitinib treatment.

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Conflict of interest statement

A.I., K.N. and N.Y. have received honoraria from Astra Zeneca. T.M. has received honoraria from Astra Zeneca and Chugai. T.N. has received honoraria from Chugai. Y.N. has received honoraria and research grants from Chugai. All other authors declare no conflicts of interest.

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CH5424802 (RO5424802) for patients with *ALK*-rearranged advanced non-small-cell lung cancer (AF-001JP study): a single-arm, open-label, phase 1–2 study

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See Comment page 564

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Summary

Background Currently, crizotinib is the only drug that has been approved for treatment of *ALK*-rearranged non-small-cell lung cancer (NSCLC). We aimed to study the activity and safety of CH5424802, a potent, selective, and orally available ALK inhibitor.

Methods In this multicentre, single-arm, open-label, phase 1–2 study of CH5424802, we recruited ALK inhibitor-naive patients with *ALK*-rearranged advanced NSCLC from 13 hospitals in Japan. In the phase 1 portion of the study, patients received CH5424802 orally twice daily by dose escalation. The primary endpoints of the phase 1 were dose limiting toxicity (DLT), maximum tolerated dose (MTD), and pharmacokinetic parameters. In the phase 2 portion of the study, patients received CH5424802 at the recommended dose identified in the phase 1 portion of the study orally twice a day. The primary endpoint of the phase 2 was the proportion of patients who had an objective response. Treatment was continued in 21-day cycles until disease progression, intolerable adverse events, or withdrawal of consent. The analysis was done by intent to treat. This study is registered with the Japan Pharmaceutical Information Center, number JapicCTI-101264.

Findings Patients were enrolled between Sept 10, 2010, and April 18, 2012. The data cutoff date was July 31, 2012. In the phase 1 portion, 24 patients were treated at doses of 20–300 mg twice daily. No DLTs or adverse events of grade 4 were noted up to the highest dose; thus 300 mg twice daily was the recommended phase 2 dose. In the phase 2 portion of the study, 46 patients were treated with the recommended dose, of whom 43 achieved an objective response (93·5%, 95% CI 82·1–98·6) including two complete responses (4·3%, 0·5–14·8) and 41 partial responses (89·1%, 76·4–96·4). Treatment-related adverse events of grade 3 were recorded in 12 (26%) of 46 patients, including two patients each experiencing decreased neutrophil count and increased blood creatine phosphokinase. Serious adverse events occurred in five patients (11%). No grade 4 adverse events or deaths were reported. The study is still ongoing, since 40 of the 46 patients in the phase 2 portion remain on treatment.

Interpretation CH5424802 is well tolerated and highly active in patients with advanced *ALK*-rearranged NSCLC.

Funding Chugai Pharmaceutical Co, Ltd.

Introduction

A fusion tyrosine kinase gene comprising the *EML4* gene and the *ALK* gene has been identified in non-small-cell lung cancer (NSCLC) with inversion of chromosome 2p. Mouse 3T3 fibroblasts expressing *EML4-ALK* had increased transforming activity and tumorigenicity.[1] Transgenic mice expressing *EML4-ALK* fusion gene in lung alveolar epithelial cells were generated and exhibited development of adenocarcinoma in lungs shortly after birth,[2] suggesting that the *EML4-ALK* fusion gene could be a driver mutation for NSCLC and serve as a promising candidate for a therapeutic target.[1,3] Therefore, the introduction of new ALK inhibitors is expected to improve the treatment of patients with *ALK*-rearranged NSCLC.[3]

So far, crizotinib, a multi-targeted receptor tyrosine kinase inhibitor of *ALK*, *MET*, and *ROS1* oncogene,[4,5] is the only agent that has been approved for *ALK*-rearranged NSCLC in the USA, European Union, Japan, and other countries. In the phase 1 trial of crizotinib in patients with *ALK*-rearranged NSCLC, 87 of 143 evaluable patients had an objective response (60·8%, 95% CI 52·3–68·9). Median progression-free survival (PFS) was 9·7 months.[6] In a retrospective study[7] comparing survival outcomes in crizotinib-treated patients enrolled in the phase 1 trial and crizotinib-naive controls screened during the same period, crizotinib therapy was associated with better survival. However, resistance to crizotinib occurs by a number of mechanisms, including *ALK* gene alterations, such as *ALK* point mutations and copy number gain, and activation of bypass signalling through activation of other oncogenes.[8,9] Additionally, poor penetration of crizotinib across the blood–brain barrier is thought to be associated with a higher incidence of brain involvement if relapse occurs.[10] In the crizotinib phase 2 trial, the most common site for single organ disease progression was the brain.[11]

CH5424802 (RO5424802; Chugai Pharmaceutical Co, Ltd, Tokyo, Japan) is a novel, highly selective oral ALK inhibitor. In-vitro kinase assays showed that this compound selectively inhibits ALK. CH5424802 also shows high anti-tumour activity both in vitro and in vivo against tumour cell lines with some type of *ALK* gene alteration, such as NSCLC and anaplastic large-cell lymphoma lines harbouring an *ALK* fusion gene and a neuroblastoma line harbouring amplified *ALK* gene. More importantly, CH5424802 yielded potential anti-tumour activity against the gatekeeper Leu1196Met mutation in *EML4-ALK*,[12] which has been identified in tumour cells refractory to crizotinib.[13]

We report the results of a phase 1–2 study of CH5424802 (AF-001JP study) that was designed to identify the maximum tolerated dose (MTD) and pharmacokinetic parameters of the drug, and subsequently to assess its activity and safety in ALK inhibitor-naive patients with *ALK*-rearranged NSCLC.

Methods

Study design and patients

This study was a multicentre, single-arm, open-label, phase 1–2 trial (AF-001JP). Patients were eligible if they were aged 20 years or older; had histologically or cytologically confirmed advanced or metastatic *ALK*-rearranged stage IIIB, IV, or recurrent NSCLC; had an Eastern Cooperative Oncology Group performance status (ECOG PS) of 0 or 1; had measurable lesions as defined by Response Evaluation Criteria in Solid Tumors (RECIST; version 1.1) (for the phase 2 portion only); received two or more (phase 1 portion) or one or more (phase 2 portion) previous chemotherapy regimens; and had adequate haematological, hepatic, and renal function. We excluded patients who had received previous treatment with any ALK inhibitor. Other exclusion criteria included symptomatic brain metastases or brain metastases requiring treatment, history of serious cardiac dysfunction, clinically significant gastrointestinal abnormality that would affect the absorption of the study drug, and pregnant or lactating women.

To identify whether patients were positive for *ALK* fusion gene expression, formalin-fixed paraffin-embedded sections from previous diagnostic or surgical procedures were sent to the laboratory in the Cancer Institute, Japanese Foundation for Cancer Research, Tokyo, Japan, and screened using anti-ALK immunohistochemistry with iAEP method (ALK Detection Kit, Nichirei Bioscience, Tokyo, Japan).[14–16] In patients who were positive by immunohistochemistry, the fluorescence in-situ hybridisation (FISH) test was subsequently done for confirmation. An experienced pathologist (KT) judged these tests. Additionally, we did a multiplex RT-PCR method (SRL, Tokyo, Japan) on samples of cells or frozen cancer tissue sections. We deemed patients to be positive for *ALK* fusion gene expression when either FISH or RT-PCR showed positive results.

In this study, patients gave written informed consent for *ALK* assessment by a central laboratory. If tumours were confirmed to be *ALK* positive, patients signed another informed consent form for enrolment into this trial. Patients participating in the study were treated at 13 hospitals in Japan. The study was approved by the institutional review board at each participating institution, and done in accordance with the Declaration of Helsinki and Good Clinical Practices.

Procedures

In the phase 1 portion of this study, patients received CH5424802 orally twice daily (once in the morning and once in the evening) in an open-label, sequential-cohort, dose-escalation study. We did the dose escalation with an accelerated titration design[17] under fasting conditions from 20 mg to 300 mg twice daily. We determined a dose of 300 mg twice daily as the highest planned dose on the basis of the available safety information about the additive formulation in Japan. Patients fasted for 2 h before administration and 1 h after administration. We predefined dose-limiting toxicities (DLTs) as a treatment-related adverse event that occurs during the DLT assessment period (from day 1 to day 3 in cycle 0 and from day 1 to day 21 in cycle 1) and met any of the following criteria: grade 4 thrombocytopenia, grade 4 neutropenia continuing for 4 days or more, non-haematological toxic effects of grade 3 or worse (excluding transient electrolyte abnormalities and diarrhoea, nausea, or vomiting that recovers to grade 2 or lower with appropriate treatment), and events that required suspension of treatment for at least 7 days. The recommended dose was to be determined after taking into consideration tumour response in addition to the MTD, safety, and pharmacokinetic parameters under fasting conditions. While this fasting part was ongoing with DLT assessment in the cohort of patients given 300 mg twice daily, we amended the study to conduct a non-fasting part at doses of 240 mg and 300 mg twice daily by a traditional 3+3 design. We assessed the effect of food by comparing results under fasting and non-fasting conditions at both doses in the two groups of patients.

In the phase 2 portion of this study, patients received CH5424802 at the recommended dose identified in the phase 1 portion of the study orally twice a day (once in the morning and once in the evening). The patients fasted for 2 h before administration and 1 h after administration. Treatment was continued in 21-day cycles until disease progression, intolerable adverse events, or withdrawal of consent.

Tumours were assessed every cycle until four cycles and every two cycles thereafter, with RECIST version 1.1. In the phase 2 portion, tumour assessment from brain to pelvis at baseline was mandatory. Tumour assessment in this trial was done with CT scans for chest and abdomen; with CT or MRI for head, neck, and pelvis; and with bone scintigraphy, PET, x-ray, CT, or MRI for bone. Adverse

events were monitored up to the 28th day after the final dose, and assessed according to the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE version 4.0). When vision disorders occurred during this trial, an ophthalmological examination was done.

If a patient had thrombocytopenia or neutropenia of grade 4 or a non-haematological toxic effect of grade 3 or higher occurred, treatment with CH5424802 would be suspended until the toxic effects improved to grade 1 or lower, or the baseline grade. If the period of suspension was 14 days or less, treatment with CH5424802 could be resumed at the same dose level. If the period of suspension was longer than 14 days, treatment with CH5424802 would be resumed at a reduced dose. Treatment with CH5424802 would be discontinued permanently if treatment could not be resumed within 21 days of suspension. Additionally to these criteria, at the initiation of every cycle, treatment with CH5424802 would commence after it had been confirmed that all the following criteria were met (neutrophil count ≥1500 cells per μL [this criterion was amended so that patients with a neutrophil count ≥1000 cells per μL could receive the next cycle of treatment], platelet count ≥$7·5\times10^4$ cells per μL; non-haematological toxic effects of grade ≤1 or grade at baseline with exception of investigator's judgment).

	Phase 1 (n=24)	Phase 2 (n=46)
Age, years	42·5 (28–67, 39·0–60·0)	48·0 (26–75, 37·5–54·5)
Sex		
Female	13 (54%)	24 (52%)
Male	11 (46%)	22 (48%)
Smoking status		
Never	14 (58%)	27 (59%)
Former	10 (42%)	18 (39%)
Present	0	1 (2%)
Histological findings*		
Adenocarcinoma	22 (92%)	46 (100%)
Squamous-cell carcinoma	1 (4%)	0
Large-cell carcinoma	1 (4%)	0
Clinical stage (at screening)		
IIIB	0	2 (4%)
IV	14 (58%)	31 (67%)
Postoperative recurrence	10 (42%)	13 (28%)
ECOG performance status		
0	9 (38%)	20 (43%)
1	15 (63%)	26 (57%)
ALK diagnosis†		
Immunohistochemistry and FISH	22 (92%)	39 (85%)
RT-PCR	2 (8%)	7 (15%)
EGFR status*		
Wild-type	22 (92%)	41 (89%)
Mutation	0	0
Unknown	2 (8%)	5 (11%)
Previous chemotherapy regimens for metastatic disease		
0	0	1 (2%)‡
1	1 (4%)‡	21 (46%)
2	10 (42%)	9 (20%)
≥3	13 (54%)	15 (33%)

Data are median (range, IQR) or number of patients (%). ECOG=Eastern Cooperative Oncology Group. FISH=fluorescence in-situ hybridisation. *Histological findings and EGFR status were reported by the investigator site. †ALK diagnosis was performed in two central reference laboratories (one for immunohistochemistry and FISH, and the other for RT-PCR). ‡Regarded as eligible for inclusion because relapse occurred within 6 months of completion of adjuvant chemotherapy.

Table 1: **Demographics and baseline characteristics**

Pharmacokinetics

In the phase 1 portion of the study, we obtained 2 mL blood samples at pre-dose, 0·5 h, 1 h, 2 h, 4 h, 6 h, 8 h, 10 h, 24 h, 32 h, 48 h, and 72 h after single oral administration of CH5424802, and at pre-dose, 0·5 h, 1 h, 2 h, 4 h, 6 h, 8 h, and 10 h at steady state under fasting and non-fasting conditions. The blood samples were centrifuged at 1500–2000×g for 10 min at 4°C. The plasma samples were then stored at –70°C or less. We measured drug concentrations in plasma by the liquid chromatography-mass spectrometry and liquid chromatography-tandem mass spectrometry with limit of quantitation of 0·1 ng/mL.

Statistical analysis

The primary endpoint of the phase 1 portion was DLT, MTD, safety, and pharmacokinetic parameters. The primary endpoint of the phase 2 portion was the proportion of patients who had an objective response, as determined by an independent review committee, which was to be confirmed by a subsequent scan. Secondary endpoints included safety, the proportion of patients who achieved disease control, progression-free survival, overall survival, and pharmacokinetic parameters.

In the phase 1 portion of the study, we did all statistical analyses in a descriptive manner; and we thus did no formal hypothesis testing. We analysed plasma CH5424802 concentrations with Phoenix WinNonlin Version 6.2 (Pharsight Corporation, Mountain View, CA, USA). We directly obtained the maximum plasma concentrations (C_{max}) from the plasma-concentration curves for every participant. We calculated the area under the plasma concentration-time curve (AUC) for every individual using the linear log trapezoidal method as implemented in Phoenix WinNonlin.

In the phase 2 portion of this study, initially, we used a threshold response rate of 25% for reference based on the response rate of a platinum doublet regimen that is a standard treatment for NSCLC,[18] and an expected response rate of 70% based on the response rate of the patients to crizotinib.[19] Since 12 individuals are necessary to yield a statistical power of 80% with a two-sided significance of 5%, we calculated a target sample size of