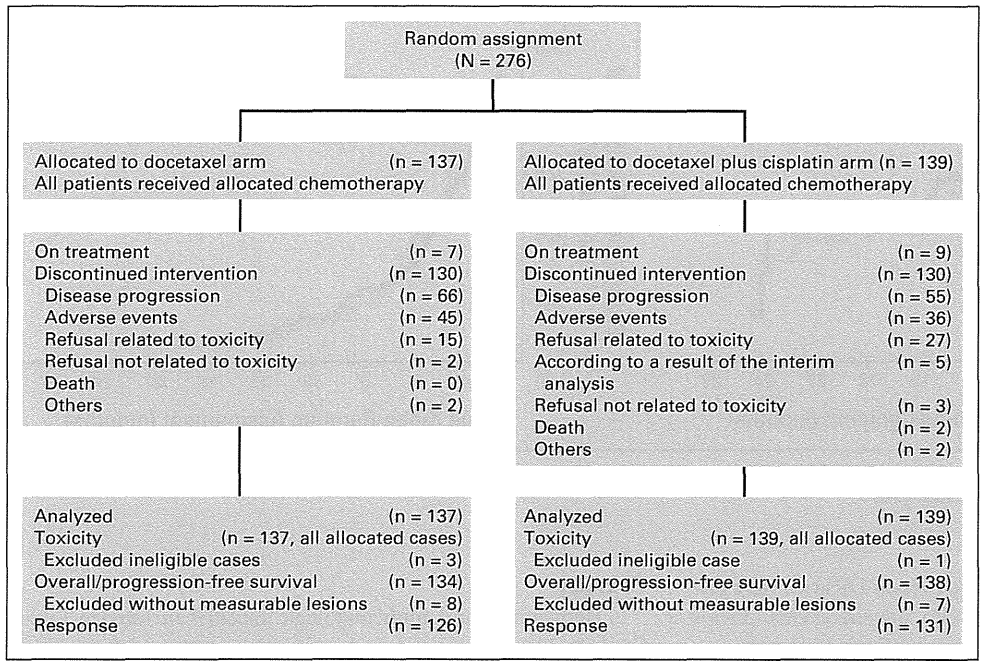

Fig 1. CONSORT diagram.

proportion of the interim events to the planned events, was 0.24 (73 of 304 events). Survival in the DP arm was inferior to that in the docetaxel arm (HR for DP to docetaxel arm, 1.56; 95% CI, 0.98 to 2.49; multiplicity-adjusted 99.99% CI, 0.62 to 3.88; one-sided $P = .97$ and two-sided $P = .06$ by stratified log-rank test), and the predictive probability that DP would be statistically superior to docetaxel on final analysis was 0.996% (< 1%). These results led to early study termination based on the recommendation of the Data and Safety Monitoring Committee, in accordance with the stopping guidelines prespecified in the protocol.

Patient Characteristics

Between October 2008 and September 2010, 276 patients (215 patients from JCOG and 61 patients from WJOG) were enrolled from 56 institutions (36 institutions affiliated with JCOG and 20 institutions affiliated with WJOG). Of these patients, 137 and 139 patients were assigned to the docetaxel and DP arms, respectively. All patients received the study treatments; therefore, all 276 patients were included in the safety analysis set. Three patients in the docetaxel arm and one patient in the DP arm were ineligible because of uncontrolled diabetes (ie, dependence on insulin injections) or previous malignancy. Therefore, these patients were excluded from survival analyses (Fig 1). Although the proportions of female patients and patients with adenocarcinoma were slightly higher in the docetaxel arm than in the DP arm, the patients' baseline characteristics were generally well balanced between the treatment arms (Table 1).

Treatment Delivery

The median number of treatment cycles was four (range, one to 18 cycles) in the docetaxel arm and three (range, one to six cycles) in the DP arm, and the proportion of patients in whom treatment continued for five or more cycles was higher in the docetaxel arm than in the DP arm (31% *v* 8%, respectively). In the docetaxel and DP arms, 37% and 4% of patients required one-step dose reductions, respectively. Furthermore, 19% of patients required two-step dose reductions in the docetaxel arm. In the DP arm, 19% of patients had one or more skipped treatments on day 8 or 15. The major reasons for

Table 1. Patient Demographics and Clinical Characteristics

Demographic or Clinical Characteristic	Docetaxel (n = 137)		Docetaxel/Cisplatin (n = 139)	
	No. of Patients	%	No. of Patients	%
Age, years				
Median	76		76	
Range	70-87		70-86	
< 75	31	23	32	23
≥ 75	106	77	107	77
Sex				
Male	95	69	101	73
Female	42	31	38	27
Smoking status*				
Never	38	28	36	26
Smoker	98	72	101	74
ECOG PS				
0	50	36	48	35
1	87	64	91	65
Stage				
III	42	31	43	31
IV or recurrence	95	69	96	69
Histology*				
Adenocarcinoma	91	67	86	63
Squamous	32	24	39	28
Others	13	10	12	9

Abbreviation: ECOG PS, Eastern Cooperative Oncology Group performance status.
*Data for one patient in the docetaxel monotherapy arm and two patients in the docetaxel plus cisplatin arm were missing.

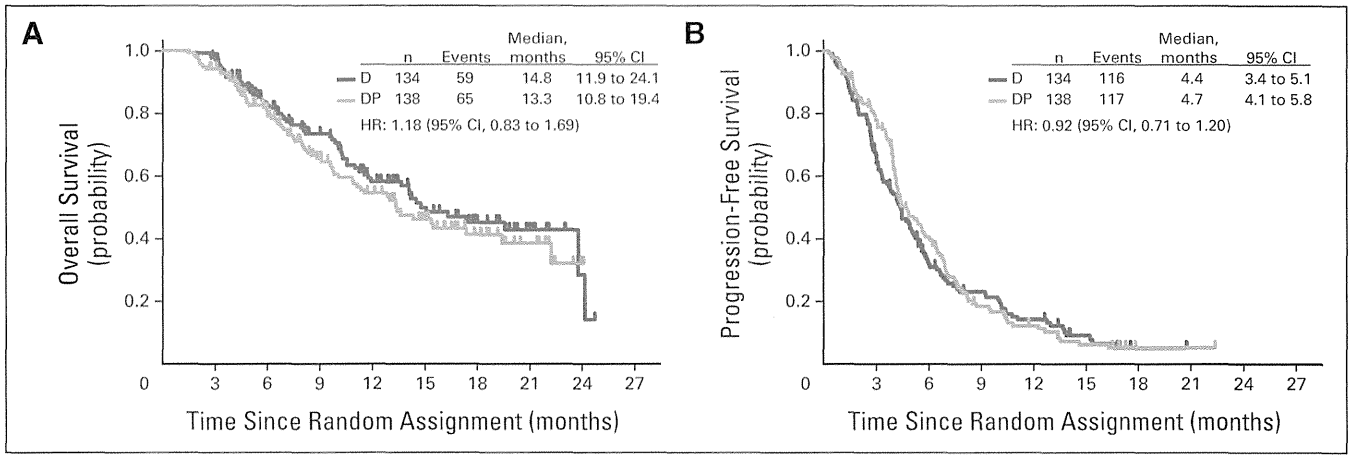

Fig 2. Kaplan-Meier curves for (A) overall survival and (B) progression-free survival. Tick marks indicate censored patients at the data cutoff point (November 2010). D, docetaxel; DP, docetaxel plus cisplatin; HR, hazard ratio.

treatment discontinuation in the docetaxel versus DP arms were disease progression (51% *v* 42%, respectively), adverse events (35% *v* 28%, respectively), and patient refusal to continue treatment as a result of toxicity (12% *v* 21%, respectively).

Efficacy

The overall RRs were 24.6% in the docetaxel arm (95% CI, 17.4% to 33.1%) and 34.4% in the DP arm (95% CI, 26.3% to 43.2%). The difference was not statistically significant ($P = .10$).

By November 22, 2010, 124 (45.6%) of the 272 eligible patients had died (docetaxel arm, n = 59; DP arm, n = 65). The median follow-up time for all eligible patients was 9.6 months. The 1-year survival rates were 58.2% and 54.5% in the docetaxel and DP arms, respectively. The HR for OS was 1.18 (95% CI, 0.83 to 1.69; Fig 2A). The HR for PFS was 0.92 (95% CI, 0.71 to 1.20; Fig 2B).

Toxicity

Hematologic and nonhematologic toxicities are listed in Table 2. Grade ≥ 3 leukopenia and neutropenia occurred more frequently in the docetaxel arm. The incidence of grade 4 neutropenia was 67.9% in the docetaxel arm but only 0.8% in the DP arm. Febrile neutropenia was observed only in the docetaxel arm at an incidence of 15.2%. Grade ≥ 3 anemia, hyponatremia, and anorexia were observed in more than 10% of patients in the DP arm. Four treatment-related deaths occurred, all in the DP arm (2.9%), including three patients who died of pneumonitis and one patient who died of unclassified sudden death.

QOL

Symptom score questionnaire responses were collected from 271 (98.2%) of 276 patients at baseline, 258 patients (93.5%) after the second cycle, and 247 patients (89.5%) after the third cycle. The

Table 2. Toxicities

	Docetaxel (n = 137)			Docetaxel/Cisplatin (n = 139)		
Adverse Event	Grade 3 or 4 (%)	Grade 4 (%)	Missing (No.)	Grade 3 or 4 (%)	Grade 4 (%)	Missing (No.)
Hematologic*						
Leukopenia	62.7	8.2	3	5.4	0	10
Neutropenia	88.8	67.9	3	10.1	0.8	10
Anemia	3.7	0.7	3	16.3	0.8	10
Thrombocytopenia	0	0	3	0.8	0	10
Nonhematologic*						
Febrile neutropenia	15.2	0	5	0	0	8
Hyponatremia	5.2	0.7	3	14.7	0.8	10
Hypoalbuminemia	1.5	—	6	4.7	—	10
Infection	7.6	0	5	8.4	0.8	8
Anorexia	1.5	0	5	10.7	0	8
Nausea	0.8	0	5	3.8	0	8
Diarrhea	3.8	0	5	0.8	0	9
Fatigue	3.0	0	5	5.3	0	8
Pneumonitis	5.3	0	5	2.3	0.8	8

NOTE. There were four treatment-related deaths (2.9%), all in the docetaxel plus cisplatin arm, including three deaths resulting from pneumonitis and one unclassified sudden death.
*Each value was calculated while excluding patients with missing data.

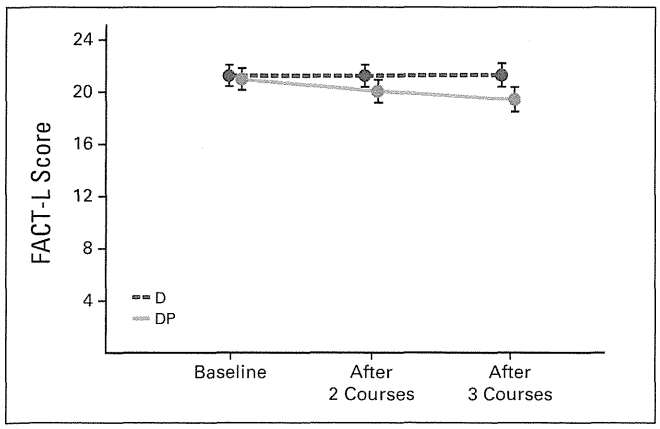

Fig 3. Quality-of-life assessments according to the seven-item Functional Assessment of Cancer Therapy–Lung (FACT-L). Dots and error bars indicate the least squared mean total scores and 95% CI, respectively. Higher scores indicate a better quality of life. D, docetaxel; DP, docetaxel plus cisplatin.

numbers of patients with missing data because of death or severe deterioration of the patient's general condition in the docetaxel and DP arms were one and six patients, respectively, after the second cycle and six and nine patients, respectively, after the third cycle. In the docetaxel and DP arms, 39.3% (53 of 135 patients) and 36.8% (50 of 136 patients) of patients had scores that improved from baseline to the end of the third cycle, which did not constitute a significant difference. Although the mean total score remained near its baseline value in the docetaxel arm, it declined gradually in the DP arm, changing in a statistically significant manner between baseline and cycle 3 ($P < .01$; Fig 3).

Supplementary Ad Hoc Analysis

Data forms were collected from 275 patients (except one patient from the docetaxel arm). *EGFR* mutation testing was performed in 79 patients (58%) and 74 patients (53%) in the docetaxel and DP arms, respectively; the results revealed active *EGFR* mutations in 22 patients in the docetaxel arm (16% overall and 28% of those tested) and 16 patients in the DP arm (12% overall and 22% of those tested). After protocol treatment completion, further drug treatment was administered to 74 patients (54%) in the docetaxel arm and 70 patients (50%) in the DP arm. During this treatment, EGFR tyrosine kinase inhibitor was administered to 35 patients (26%) and 23 patients (17%) in the docetaxel and DP arms, respectively.

Figure 4 shows the survival HRs according to subgroup analyses of the baseline and ad hoc characteristics. No significant differences between the two treatment groups were observed in any subgroup.

DISCUSSION

The standard treatment for fit patients with advanced NSCLC is platinum-doublet chemotherapy.[6,7] Several retrospective subgroup analyses have shown that platinum-doublet chemotherapy is similarly effective in elderly and younger patients and is well tolerated despite an increased incidence of toxicity.[9,10] These retrospective analyses, however, were performed in highly selected elderly populations. Generally, elderly patients are often unsuitable candidates for bolus cisplatin administration because of comorbid illnesses and/or organ dysfunction. Therefore, we considered it important to conduct a prospective investigation to determine whether the addition of a modified platinum agent might improve survival in elderly patients with NSCLC.

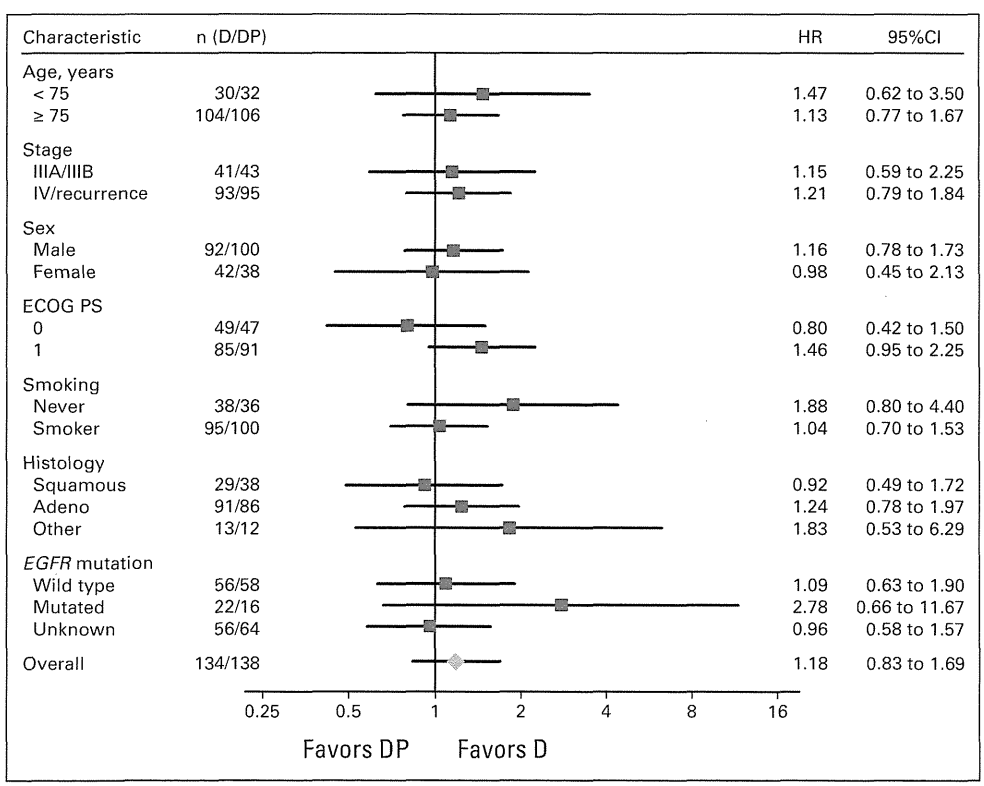

Fig 4. Subgroup analysis of overall survival. D, docetaxel; DP, docetaxel plus cisplatin; ECOG PS, Eastern Cooperative Oncology Group performance status; HR, hazard ratio.

In the phase II and previous phase III trials, we demonstrated that weekly split docetaxel and additional cisplatin reduced myelotoxicity and increased RRs.[13,14] In this study, we analyzed the add-on effect of weekly cisplatin over docetaxel monotherapy. Although the DP arm tended to have higher RRs than the docetaxel arm, this was reflected in neither the PFS nor the OS.

Although we collected information on comorbid illnesses, we did not assess the Charlson comorbidity index. Comprehensive geriatric assessments, including basic activities of daily living (ADLs), instrumental ADLs, Mini-Mental State Examination, and Geriatric Depression Scale evaluation, were also conducted for exploratory purposes. Although the prognostic values of these assessments have not been validated for elderly patients with lung cancer, it was suggested that ADLs and Mini-Mental State Examination can be useful.[18] In future research, we should evaluate these factors prospectively.

The proportions of female patients and patients with adenocarcinoma were slightly higher in the docetaxel arm than in the DP arm. In eastern Asia, including Japan, active *EGFR* mutations are often observed in such patients and have been reported as a favorable prognostic factor in patients with NSCLC.[19,20] According to a subgroup analysis, the median survival time was 12.8 months in the 114 patients (in the docetaxel plus DP arms) without *EGFR* mutation and 24.1 months in the 38 mutation-positive patients. The proportion of patients with active *EGFR* mutations was slightly higher in the docetaxel arm than in the DP arm. However, it would have been difficult to demonstrate the superiority of the DP arm in OS, considering the slight difference in PFS, even if there were no such imbalances.

In the docetaxel arm, a higher proportion of patients required dose reductions, yet these appropriate reductions lengthened treatment. In contrast, the DP arm included fewer patients who were able to continue treatment, despite the lower proportion of dose reductions and skipped treatments. We believe that declining QOL was an important cause of treatment discontinuation in the DP arm.

The toxicity profiles also differed between the two arms. In the docetaxel arm, neutropenia was most prominent, and grade 4 neutropenia occurred in up to 68% of the patients. Consequently, febrile neutropenia was observed in 15% of the patients in the docetaxel arm, whereas no patients experienced febrile neutropenia in the DP arm. The frequency of febrile neutropenia in the docetaxel arm was similar to that seen in a previous Japanese docetaxel study for elderly patients.[5] However, because febrile neutropenia was successfully managed with appropriate supportive treatments, there were no treatment-related deaths in the docetaxel arm. However, the DP arm had higher incidences of grade ≥ 3 anemia, hyponatremia, and anorexia. We suppose that these were the main causes of the decline in the QOL score in the DP arm. The median number of treatment cycles and the proportion of patients in whom treatment could be continued for five or more cycles in the DP arm were smaller than those in the docetaxel arm. These findings could be associated with the decline in QOL and might have affected OS in the DP arm. Three of four treatment-related deaths in the DP arm were caused by pneumonitis. It was reported that weekly docetaxel administration increases the frequency of pneumonitis.[21,22] In this study, there were few differences in the frequencies of pneumonitis between the two arms; however, more severe pneumonitis was observed in the DP arm.

Quoix et al[18] demonstrated the superiority of carboplatin plus weekly paclitaxel over conventional standard therapy, namely vinorelbine or gemcitabine monotherapy, in the Intergroupe Francophone de Cancerologie Thoracique 0501 study. The usefulness of platinum-based treatments in elderly patients was first shown in a prospective study. For elderly patients with NSCLC, carboplatin combination therapy may be preferable to a split cisplatin combination. However, the high incidence of toxicity could not be ignored, because treatment-related deaths occurred in 4.4% of patients in the doublet arm but only in 1.3% of patients in the monotherapy arm.[18] In contrast, a phase I trial of combined carboplatin plus pemetrexed (PEM), followed by maintenance PEM, showed good tolerability in elderly patients with nonsquamous NSCLC.[23] We consider that the combination of carboplatin plus PEM should be compared with docetaxel monotherapy.

In conclusion, this study failed to demonstrate any advantages of weekly DP over docetaxel monotherapy as first-line chemotherapy for elderly patients with advanced NSCLC, and docetaxel every 3 weeks remains the standard treatment for elderly patients with advanced NSCLC.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Although all authors completed the disclosure declaration, the following author(s) and/or an author's immediate family member(s) indicated a financial or other interest that is relevant to the subject matter under consideration in this article. Certain relationships marked with a "U" are those for which no compensation was received; those relationships marked with a "C" were compensated. For a detailed description of the disclosure categories, or for more information about ASCO's conflict of interest policy, please refer to the Author Disclosure Declaration and the Disclosures of Potential Conflicts of Interest section in Information for Contributors.
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GLOSSARY TERMS

cisplatin: an inorganic platinum agent (cis-diamminedichloroplatinum) with antineoplastic activity. Cisplatin forms highly reactive, charged, platinum complexes, which bind to nucleophilic groups such as GC-rich sites in DNA, inducing intrastrand and interstrand DNA cross-links as well as DNA-protein cross-links. These cross-links result in apoptosis and cell growth inhibition. Carboplatin and oxaliplatin are other members of this class.

docetaxel: a member of the taxane group of antimitotic chemotherapy medications whose mode of action is to bind and stabilize microtubules and thus disrupt cell division.

non–small-cell lung cancer (NSCLC): a type of lung cancer that includes squamous cell carcinoma, adenocarcinoma, and large-cell carcinoma.

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Appendix

Reasons for Bolus Cisplatin Administration Unsuitability

Patients age 70 to 74 years were examined before enrollment for the following six conditions, which defined them as unsuitable for bolus cisplatin administration (Appendix Table A1): a combination of more than one mild organ dysfunction, but violating none of the inclusion criteria; a combination of comorbid illness and mild organ dysfunction, but violating none of the inclusion criteria; organ dysfunction not specified by the inclusion/exclusion criteria; a combination of more than one comorbid illness; a comorbid illness not specified by the exclusion criteria; or any other condition.

Procedures of Administration

In the docetaxel monotherapy arm, docetaxel was diluted with 250 to 500 mL of 5% glucose solution or physiologic saline and administered by intravenous infusion over 60 minutes.

In the docetaxel plus cisplatin (DP) arm, docetaxel was diluted with 250 mL of 5% glucose solution or 200 mL of physiologic saline and administered by intravenous infusion over 60 minutes. Cisplatin was administered by intravenous infusion over 15 to 20 minutes, directly or after being diluted with physiologic saline, after docetaxel administration. A total of 1,000 to 1,500 mL of fluid was administered before and after the administration of cisplatin. During treatment with cisplatin, careful attention was paid to urinary output, and diuretics such as mannitol and furosemide were administered if necessary. Antiemetics such as 5-hydroxytryptamine-3 receptor antagonists and steroids were also administered if necessary.

Dose Reduction Criteria and Methods

In both arms, the presence of grade 4 neutropenia, febrile neutropenia, or grade $\geq$ 3 nonhematologic toxicity (except anorexia, nausea, vomiting, hyponatremia, constipation, and hyperglycemia) necessitated dose reduction (docetaxel arm levels -1 and -2: docetaxel 50 and 40 mg/m^2, respectively; DP arm level -1: docetaxel 15 mg/m^2 and cisplatin 20 mg/m^2). In addition, if serum creatinine levels exceeded 2.0 mg/dL, the administration of cisplatin was stopped in subsequent cycles in the DP arm. The persistence of these toxicities after two dose-reduction steps in the docetaxel arm or one dose-reduction step of each drug in the DP arm prompted treatment discontinuation.

Definition of Overall and Progression-Free Survival

Overall survival was measured from the date of random assignment to death from any cause and was censored at the last follow-up date. Progression-free survival was measured from the date of random assignment to the first observation of disease progression or death from any cause if there was no progression. If there was no progression and the patient did not die, progression-free survival data were censored at the date on which the absence of progression was confirmed.

Table A1. Conditions Defining Patients As Unsuitable for Bolus Cisplatin Administration

Condition	No. of Patients	
	Docetaxel (n = 31)	Docetaxel/Cisplatin (n = 32)
Combination of more than one mild organ dysfunction, but violating none of the inclusion criteria	6	4
Combination of comorbid illness and mild organ dysfunction, but violating none of the inclusion criteria	5	8
Organ dysfunction not specified by the inclusion/exclusion criteria	8	3
Combination of more than one comorbid illness	1	7
Comorbid illness not specified by the exclusion criteria	2	2
Any other condition	9	8

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Phase I study of the HER3-targeted antibody patritumab (U3-1287) combined with erlotinib in Japanese patients with non-small cell lung cancer☆

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ABSTRACT

Objectives: Human epidermal growth factor receptor 3 (HER3) is a key dimerization partner for HER family members and is associated with resistance to other HER family receptor-targeted therapeutics. This study evaluated the safety, tolerability, pharmacokinetics and efficacy of patritumab (U3-1287), a fully human anti-HER3 monoclonal antibody, in combination with erlotinib, an epidermal growth factor receptor-tyrosine kinase inhibitor in patients with previously treated advanced non-small cell lung cancer (NSCLC).

Patients and methods: This study enrolled patients with stage IIIB/IV NSCLC with Eastern Cooperative Oncology Group performance status 0–1, life expectancy >3 months and who had progressed after at least one prior course of chemotherapy (excluding erlotinib). This open-label study included two parts: dose escalation (Part 1) and dose expansion (Part 2). In Part 1, patients received intravenous patritumab 9 or 18 mg/kg every 3 weeks in addition to per-oral erlotinib 150 mg/day daily. In Part 2, patients received the recommended dose of patritumab as determined in Part 1. Adverse event rates, pharmacokinetics and tumor responses were determined.

Results: Twenty-four Japanese patients received patritumab at 9 mg/kg ($n=3$) or 18 mg/kg ($n=21$), and erlotinib. No dose-limiting toxicities were reported, indicating the maximum-tolerated dose was not reached. The most frequent adverse events were gastrointestinal or skin toxicities, which were generally mild and manageable. Patritumab pharmacokinetics were similar to those reported in previous studies. The median progression-free survival (95% confidence interval) was 44.0 (22.0–133.0) days for the *EGFR* wild-type group ($n=9$) and 107.0 (74.0–224.0) days for the *EGFR*-activating mutation group ($n=13$). Evaluation of biomarkers by immunohistochemical analysis did not indicate a relationship between efficacy and HER3 expression in tumor tissues.

Conclusion: Patritumab in combination with erlotinib was well tolerated and the efficacy of the combination was encouraging, especially in patients where prior gefitinib treatment failed.

Abbreviations: HER, human epidermal growth factor receptor; NSCLC, non-small cell lung cancer; DLT, dose-limiting toxicity; MTD, maximum tolerated dose; EGFR-TKI, epidermal growth factor receptor-tyrosine kinase inhibitor; RECIST, response evaluation criteria in solid tumors; ECOG, Eastern Cooperative Oncology Group; CTCAE, Common Terminology Criteria for Adverse Events; AE, adverse events; ELISA, enzyme-linked immunosorbent assay; AUC, area under the curve; DCR, disease control rate; ORR, overall response rate; PR, partial response; SD, stable disease; PD, progressive disease.

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1. Introduction

The human epidermal growth factor receptor (HER) family consists of four structurally related cellular receptors (Her1, Her2, Her3, Her4) that are expressed on the surface of cells and contain extracellular, transmembrane, and tyrosine kinase domains. Each of these domains is responsible for a different aspect of HER signaling pathways. Ligand binding to HERs results in the formation of homo- or heterodimers that activate receptor tyrosine kinases and subsequently downstream PI3K and AKT pathway signaling to mediate various cellular processes including morphogenesis, proliferation, angiogenesis, and survival [1]. Thus, inappropriate activation of HER signaling pathways might cause the growth and spread of cancer cells.

HER3 is the only HER family member that lacks tyrosine kinase activity because of an amino acid substitution in the conserved kinase domain. Thus, interactions of HER3 with binding partners are essential for its biological activity [2]. In particular, HER3 potently activates downstream PI3K and AKT pathway signaling by directly binding to PI3K through six consensus phosphotyrosine sites [3]. HER3 is overexpressed in various solid tumors including non-small cell lung cancer (NSCLC) [4–7], and is a poor prognostic factor as patients with these cancers have shorter survival [8–11]. *In vitro* studies have confirmed the direct involvement of HER3 in cancer cell growth [12–14]. Moreover, a recent study suggested that HER3 was involved in the development of resistance to other HER family receptor(s)-targeted therapeutics [12]. Therefore, HER3 is considered an important target for cancer chemotherapy.

Patritumab is a fully human monoclonal immunoglobulin G1 (IgG1) antibody [15] that specifically binds to the extracellular domains of HER3, thereby inhibiting downstream signal transduction and reducing HER3 expression [15]. A more recent study indicated that patritumab abrogated cetuximab resistance in colorectal cancer cells by inhibiting the phosphorylation of EGFR, HER2, HER3, ERK, and AKT [16]. In a mouse model of human NSCLC using a Calu-3 (a cell line) xenograft, administration of patritumab alone inhibited tumor proliferation. In addition, the combined use of patritumab with erlotinib, an epidermal growth factor receptor-tyrosine kinase inhibitor (EGFR-TKI), led to increased inhibition of tumor proliferation, compared with patritumab alone [17].

In a phase I study (ClinicalTrials.gov Identifier: NCT00730470), the tolerability and safety of patritumab in patients with advanced solid tumors were evaluated. No dose-limiting toxicity (DLT) was observed at doses of 0.3–20 mg/kg, the maximum tolerated dose (MTD) was not reached, and the safety of doses up to 20 mg/kg was confirmed [18]. In another phase I study (ClinicalTrials.jp Identifier: JapicCTI-101262), the tolerability and safety of patritumab at doses of 9 mg/kg and 18 mg/kg were evaluated in Japanese patients with advanced solid tumors. No DLTs were observed at the dose levels studied and the MTD was not reached [19].

Based on an *in vivo* study showing that combined patritumab and erlotinib enhanced inhibition of tumor proliferation, compared with patritumab alone [20], the current study evaluated the safety and pharmacokinetics of patritumab combined with erlotinib and determined the recommended dose for subsequent clinical studies. Anti-patritumab antibody formation, tumor responses and potential biomarkers related to patritumab were also evaluated.

2. Patients and methods

2.1. Patients

This study enrolled stage IIIB/IV NSCLC patients who had progressed after at least one prior course of chemotherapy. Patients were 20–75 years old at the time of provision of informed consent, had measurable disease as per the response evaluation criteria in solid tumors (RECIST v.1.1, Japanese version) [21], had an Eastern Cooperative Oncology Group (ECOG) performance status (PS) of 0–1 [22], and a life expectancy of more than 3 months. Additional inclusion criteria required for patients were adequate hematologic, hepatic and renal function. Eligible patients must have recovered from any toxicity related to prior therapy, except for alopecia. Exclusion criteria included a history of erlotinib or anti-HER3 therapy (prior gefitinib therapy allowed); other active malignancies; history or presence of interstitial lung disease; history (within 6 months before enrollment) or presence of severe cardiovascular or cerebrovascular disease, pulmonary thrombosis, deep vein thrombosis, or other clinically severe pulmonary disease; any of the following complications including clinically severe infections requiring systemic administration of an antimicrobial agent, antiviral agent or other agents; presence of chronic diarrhea, inflammatory bowel disease or partial ileus; presence of peptic ulcer; fluid retention requiring treatment; corneal disease; uncontrolled diabetes mellitus; hypertension; psychiatric symptoms; a positive test for hepatitis B virus surface antigen, hepatitis C virus antibody or human immunodeficiency virus antibody; history of a bleeding diathesis; and history of serious hypersensitivity to drugs containing polysorbate 20.

The study protocol was approved by each participating Institutional Review Board and each patient provided written informed consent.

2.2. Study design

This was a multicenter open-label trial conducted in two parts: dose escalation (Part 1) and dose expansion (Part 2). In Part 1, evaluation of the DLT for the combined treatment with patritumab and erlotinib was conducted at two dose levels: 9 mg/kg and 18 mg/kg patritumab where dose escalation followed a modified 3 + 3 design. The recommended dose of 18 mg/kg patritumab alone was previously determined in a phase I study [19]. Patients were initially enrolled in a cohort to receive patritumab 9 mg/kg (Level 1) every 3 weeks in combination with an oral daily dose of erlotinib 150 mg. Patritumab was administered as an intravenous infusion over 60 minutes. The first cycle (Day 1–21, with Day 1 defined as the first day of patritumab administration) served as the DLT evaluation period. If DLTs were observed in less than 33% of patients, the dose was escalated to 18 mg/kg (Level 2).

DLTs were defined as toxicities graded in accordance with the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE v4.0) [21] and assessed as related to either patritumab or erlotinib: (1) grade 3 or higher febrile neutropenia, or persistent (7 days or longer) grade 4 neutropenia, (2) grade 4 thrombocytopenia, or grade 3 thrombocytopenia requiring blood transfusion, (3) uncontrollable grade 3 or higher fatigue, anorexia, nausea, vomiting, skin disorder (*e.g.*, skin eruption, urticaria), and diarrhea despite maximal supportive therapy, (4) grade 3 or higher toxicity, with the exception of (1)–(3) as well as pyrexia without neutropenia, transient electrolyte abnormality, and transient laboratory abnormality not requiring treatment and without clinical symptoms, and (5) toxicity requiring suspension of erlotinib therapy for more than 7 days during the DLT evaluation period. The MTD was defined as the highest dose level in the first cycle at which the frequency of DLT was below 33%.

Part 2 was designed to assess further the safety of the combined treatment in 18 patients using the recommended dose that was determined in Part 1. The target sample size for the U3-1287 + erlotinib combination therapy arm in the phase II study (ClinicalTrials.gov Identifier: NCT01211483) was 130 subjects. We selected 21 patients for the phase II study (3 for Part 1 and 18 for Part 2) based on that being approximately 15% of the target sample size.

In addition, the incidences of anti-patritumab antibodies, tumor response and related biomarkers were assessed.

2.3. Safety evaluation

Adverse events (AEs) were evaluated according to CTCAE v4.0 throughout the treatment period until 30 days after administration of the last dose (patritumab or erlotinib). Safety evaluations were based on a medical review of AEs and the results of clinical laboratory tests, vital sign measurements, 12-lead electrocardiograms, physical examination, ECOG PS, and X-ray/computed tomography scans. The presence of anti-patritumab antibodies was assessed before each treatment cycle and measured by electrochemiluminescence immunoassay.

2.4. Pharmacokinetics

Pharmacokinetics were evaluated in 3 patients in the 9 mg/kg dose group and in 11 patients in the 18 mg/kg dose group. Blood samples were collected at pre-dose and 1 (end of infusion), 4, 7, 24 and 72 h after the start of first dose infusion, on Days 8 and 15 of Cycle 1, and on Day 1 of Cycles 2, 3 and 4. Serum patritumab concentrations were measured by enzyme-linked immunosorbent assay (ELISA). Pharmacokinetic parameters after the first dose were calculated by non-compartmental analysis using WinNonlin (Ver 5.2.1, Pharsight Corp., CA, USA). Pharmacokinetic statistical analyses were performed using SAS System Release 9.2 (SAS Institute Japan Ltd., Tokyo, Japan).

2.5. Tumor response

Tumor responses were evaluated using RECIST v 1.1, Japanese version [22]. Disease responses were assessed at screening and at the end of Cycle 2 and every 6 weeks thereafter.

2.6. Biomarkers

Analysis of biomarkers using tumor tissues was performed only for patients who had provided written informed consent to participate in biomarker research. Paraffin-embedded samples of archived tumor tissues were used to evaluate HER3 protein expression level by immunohistochemistry. The frequency of HER3 gene amplification was assessed by fluorescence *in situ* hybridization at Mosaic Laboratories (Lake Forest, CA, USA).

Serum HER3 levels were also evaluated in all patients. Blood for serum biomarkers was collected on Day 1 (before administration), 8, 15 and 21 of Cycle 1, and Day 21 of Cycle 2 and every 6 weeks thereafter, and changes in soluble HER3 serum levels were evaluated. Soluble HER3 levels were measured by ELISA.

2.7. Statistical methods

All patients who received study medication were included in the analysis of safety and efficacy. Safety and efficacy data were summarized as descriptive statistics using SAS System Release 8.2 (SAS Institute Japan Ltd., Tokyo, Japan). In this study, no significance level was established because no hypothesis test was performed.

3. Results

3.1. Patient characteristics

Of the 25 patients enrolled in this study, 1 was ineligible because of suspected radiation pneumonitis after registration and withdrew before receiving any study treatment. Therefore, the study drug was administered to 24 patients. In Part 1, 3 patients received patritumab 9 mg/kg (Level 1) and 3 patients received patritumab 18 mg/kg (Level 2); in Part 2, 18 patients received patritumab 18 mg/kg.

Table 1
Demographics and baseline characteristics.

	Patritumab 9 mg/kg $N=3$ (%)	Patritumab 18 mg/kg $N=21$ (%)	Overall $N=24$ (%)
Sex			
Male	3 (100)	13 (61.9)	16 (66.7)
Female	0	8 (38.1)	8 (33.3)
Age (years)			
Median (range)	60.0 (53–69)	67.0 (36–73)	66.5 (36–73)
ECOG PS[a]			
0	2 (66.7)	7 (33.3)	9 (37.5)
1	1 (33.3)	14 (66.7)	15 (62.5)
Histology			
Adenocarcinoma	2 (66.7)	17 (81.0)	19 (79.2)
Squamous cell	0	3 (14.3)	3 (12.5)
Large cell	0	1 (4.8)	1 (4.2)
Other	1 (33.3)	0	1 (4.2)
Stage			
IIIB	0	0	0
IV	3 (100)	21 (100)	24 (100)
EGFR genotype			
Wild-type	1 (33.3)	8 (38.1)	9 (37.5)
Mutations	1 (33.3)	12 (57.1)	13[b] (54.2)
Exon 19	1 (33.3)	6 (28.6)	7 (29.2)
L858R	0	5 (23.8)	5 (20.8)
Exon 18 and 21	0	1 (4.8)	1 (4.2)
T790M	0	0	0
Unknown	1 (33.3)	1 (4.8)	2 (8.3)
Number of prior chemotherapy lines			
Median (range)	4.0 (2–4)	2.0 (1–4)	2.5 (1–4)

[a] Eastern Cooperative Oncology Group performance status.
[b] 12 of 13 patients received prior gefitinib therapy.
EGFR, epidermal growth factor.

Patient characteristics are summarized in Table 1. Eight patients were female and 16 patients were male. The median age (range) was 66.5 (36–73) years. Tumor genotyping of *EGFR* showed wild-type *EGFR* in 9 patients (37.5%), deletion of exon 19 (Exon 19 del) in 7 patients (29.2%), substitution of amino acid arginine with leucine at 858 (L858R) in 5 patients (20.8%), deletion of exon 18 and 21 (exon 18 and 21 del) in 1 patient (4.2%), and was unknown in 2 patients (8.3%). The median (range) number of lines of prior chemotherapy was 2.5 (1–4).

3.2. Safety

Throughout the study, adverse events (AEs) were reported in all 24 patients as summarized in Table 2. The most common overall AEs (≥50%) were diarrhea, stomatitis, paronychia, dermatitis acneiform, dry skin, decreased weight, and decreased appetite, which were generally mild and manageable. Most of the AEs were related to both patritumab and erlotinib, and were generally mild and manageable. No grade 5 AEs were reported. One patient receiving patritumab 18 mg/kg had a decreased lymphocyte count, which was a grade 4 AE. Serious adverse events (SAEs) including bacterial pneumonia, abnormal hepatic function, bacterial infection, cancer pain, and acneiform rash were reported in 4 patients, and these events required hospitalization. No DLT was reported during the DLT observation window and the tested doses did not reach the MTD. No patients developed anti-patritumab antibodies after the administration of patritumab in this study.

3.3. Pharmacokinetics

Serum patritumab pharmacokinetic parameters are summarized in Table 3. For the 9 and 18 mg/kg dose groups, the mean area under the curve (AUC) values were 1190 and 2480 μg/day/mL; the

Table 2
Treatment-emergent adverse events in more than 10% of patients.

Preferred term	Patritumab 9 mg/kg (*N* = 3)		Patritumab 18 mg/kg (*N* = 21)		Overall (*N* = 24)
	≥3 *N*	Any grades *N* (%)	≥3 *N*	Any grades *N* (%)	Any grades *N* (%)
Diarrhea	0	3 (100.0)	4	20 (95.2)	23 (95.8)
Stomatitis	0	2 (66.7)	1	20 (95.2)	22 (91.7)
Paronychia	0	2 (66.7)	1	18 (85.7)	20 (83.3)
Dermatitis acneiform	0	2 (66.7)	3	15 (71.4)	17 (70.8)
Dry skin	0	2 (66.7)	0	13 (61.9)	15 (62.5)
Weight decreased	0	3 (100.0)	0	10 (47.6)	13 (54.2)
Decreased appetite	0	3 (100.0)	0	9 (42.9)	12 (50.0)
Rash maculo-papular	0	3 (100.0)	1	8 (38.1)	11 (45.8)
Nausea	0	2 (66.7)	0	8 (38.1)	10 (41.7)
Dysgeusia	0	1 (33.3)	0	8 (38.1)	9 (37.5)
Cheilitis	0	0 (0.0)	0	7 (33.3)	7 (29.2)
Vomiting	0	2 (66.7)	0	5 (23.8)	7 (29.2)
Malaise	0	3 (100.0)	0	4 (19.0)	7 (29.2)
Blood bilirubin increased	1	1 (33.3)	0	6 (28.6)	7 (29.2)
Alanine aminotransferase increased	0	0 (0.0)	1	6 (28.6)	6 (25.0)
Hypoalbuminemia	0	0 (0.0)	0	5 (23.8)	5 (20.8)
Pruritus	0	0 (0.0)	0	5 (23.8)	5 (20.8)
Fatigue	0	0 (0.0)	0	5 (23.8)	5 (20.8)
Aspartate aminotransferase increased	0	0 (0.0)	0	5 (23.8)	5 (20.8)
Lymphocyte count decreased	0	0 (0.0)	1	4 (19.0)	4 (16.7)
Anemia	0	0 (0.0)	0	3 (14.3)	3 (12.5)
Hypertriglyceridemia	0	1 (33.3)	0	2 (9.5)	3 (12.5)
Headache	0	0 (0.0)	0	3 (14.3)	3 (12.5)
Dry eye	0	1 (33.3)	0	2 (9.5)	3 (12.5)
Cough	0	1 (33.3)	0	2 (9.5)	3 (12.5)
Abdominal pain upper	0	1 (33.3)	0	2 (9.5)	3 (12.5)
Constipation	0	1 (33.3)	0	2 (9.5)	3 (12.5)
Proteinuria	0	0 (0.0)	0	3 (14.3)	3 (12.5)
Blood alkaline phosphatase increased	0	0 (0.0)	0	3 (14.3)	3 (12.5)
Blood uric acid decreased	0	0 (0.0)	0	3 (14.3)	3 (12.5)

Table 3
Pharmacokinetic parameters after intravenous infusion of patritumab.

Parameters	Unit	Patritumab 9 mg/kg (*N* = 3) mean ± SD	Patritumab 18 mg/kg (*N* = 11) mean ± SD
$AUC_{0\text{-}21day}$	μg day/mL	1190 ± 87.6	2480 ± 420
C_{max}	μg/mL	242 ± 29.4	400 ± 46.7
CL	mL/day/kg	6.94 ± 0.72	6.61 ± 1.08
V_{ss}	mL/kg	51.4 ± 2.97	58.0 ± 14.8
$T_{1/2}$	day	6.44 ± 1.20	7.12 ± 2.30

AUC, area under the curve; C_{max}, maximum concentration; CL, total body clearance; V_{ss}, apparent volume of distribution at steady state; $T_{1/2}$; half-life.

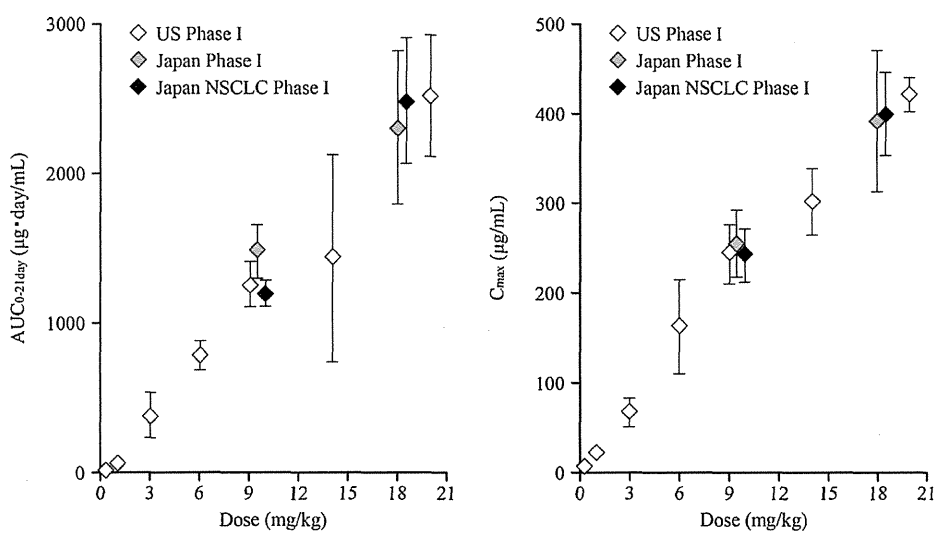

Fig. 1. C_{max} and AUC for patritumab in Phase I studies in Japan and the US. NSCLC: non-small cell lung cancer.

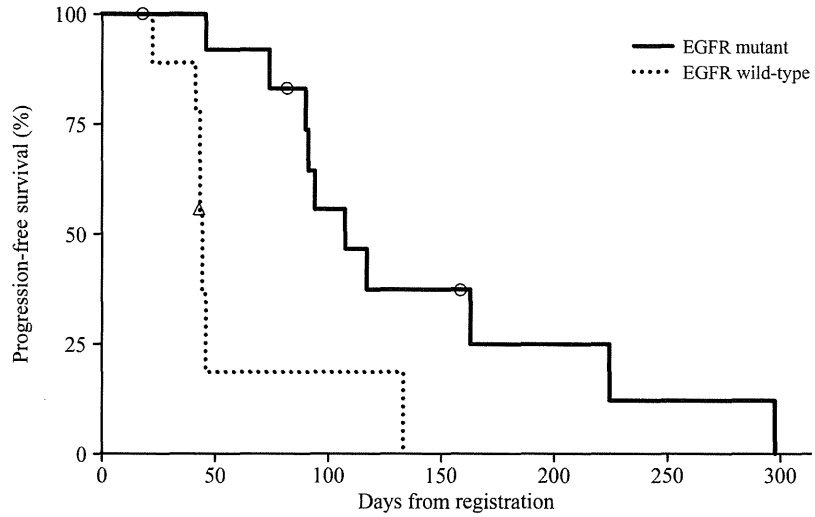

Fig. 2. Kaplan–Meier analysis of progression-free survival: subgroup analysis by *EGFR* status. EGFR: epidermal growth factor receptor.

Table 4
HER3 levels in tumor samples, *EGFR* status and tumor responses in NSCLC patients.

HER3 expression (IHC: membrane)	Total $N=11$	*EGFR* status		Best response		
		Mutant $N=7$	Wild-type $N=4$	PR $N=1$	SD $N=5$	PD $N=5$
3+	0	0	0	0	0	0
2+	6	5	1	1	3	2
1+	1	1	0	0	1	0
0	4	1	3	0	1	3

IHC, immunohistochemistry; EGFR, epidermal growth factor receptor; NSCLC, non-small cell lung cancer; PR, partial response; SD, stable disease; PD, progressive disease.

C_{max} values were 242 and 400 μg/mL; and the terminal half-lives were 6.44 and 7.12 days, respectively. The AUC and C_{max} values for patritumab are shown in Fig. 1. The AUC and C_{max} values in this study were within a similar range to those from phase I studies in Japan and the US (Fig. 1) [18,19].

3.4. Efficacy

One partial response (PR) and 14 cases with stable disease (SD) (1 SD at level 1, and 1 PR and 13 SD at level 2) were observed at 6 weeks. The overall response rate (ORR) was 4.2% and the disease control rate (DCR = CR + PR + SD) was 62.5%.

The PR was observed in a patient who had a tumor with an *EGFR*-activating mutation (L858R) but who had received no prior EGFR-TKI. Among the 14 SD patients, 10 patients had tumors with an *EGFR*-activating mutation and received prior gefitinib treatment (exon 19 del, $n=5$; L858R, $n=4$; exon 18 and 21 del, $n=1$), 3 patients had a tumor with wild-type *EGFR* and 1 patient had a tumor with an unknown *EGFR* status without a history of prior gefitinib treatment.

The progression-free survival (PFS) is shown in Fig. 2. The median PFS (95% confidence interval) was 44.0 (22.0–133.0) days for the *EGFR* wild-type group ($n=9$) and 107.0 (74.0–224.0) days for the *EGFR*-activating mutation group ($n=13$). The median PFS (95% confidence interval) in patients who had a tumor with an *EGFR*-activating mutation and who had received prior gefitinib treatment ($n=12$) was 107.0 (74.0–224.0) days.

3.5. Biomarker analysis

Tumor tissues for biomarker identification analysis were obtained from 11 patients. HER3 protein levels, *EGFR* mutation status, and tumor responses of these patients are summarized in Table 4. A correlation between tumor response and HER3 expression was not found. Serum soluble HER3 concentrations during treatment with patritumab and erlotinib significantly increased from baseline in all patients. Soluble HER3 concentrations (mean ± SD) at baseline were 6.88 ± 0.48 ng/mL for 9 mg/kg ($n=3$) and 7.35 ± 2.48 ng/mL for 18 mg/kg ($n=21$) groups. Soluble HER3 concentrations (mean ± SD) on cycle 1 day 21 were 29.72 ± 1.14 ng/mL for the 9 mg/kg ($n=3$) group and 27.53 ± 6.17 ng/mL for the 18 mg/kg ($n=21$) group. There was no statistically significant difference in soluble HER3 concentrations between dose groups. A correlation between serum soluble HER3 concentrations during the treatment and tumor response was not found.

4. Discussion

We completed the first phase I study of patritumab in combination with erlotinib that evaluated the safety, pharmacokinetics and potential biomarkers in patients with previously treated advanced NSCLC. We found that the combination therapy had good efficacy in advanced NSCLC patients, especially for those who had tumors with *EGFR*-activating mutations and had developed resistance to gefitinib treatment.

The findings in the current study (the median PFS of patients with *EGFR*-activating mutations who received prior gefitinib treatment ($n=12$) was 3.56 months, and there were 13 patients (92.9%) with SD at level 2) compared favorably to the findings of recent studies investigating third-generation EGFR-TKIs. Early phase I results with CO-1686, a third-generation EGFR-TKI, indicated that, of 9 patients with EGFR-activating mutations and failed EGFR-TKI therapy, 2 (22.2%) had SD [23]. A study investigating another third-generation EGFR-TKI, AZD9291, demonstrated that 15 patients

(43%) had confirmed or unconfirmed PR [24]. A phase Ib study of patients with EGFR-TKI resistance treated with combined afatinib and cetuximab showed that 35% of patients responded and 95% had SD [25], demonstrating a better response than was shown in a trial using afatinib alone (response rate of 8.2%, median PFS of 4.4 months with a median overall survival of 19.0 months) [26].

A study investigating the use of cetuximab in EGFR-positive NSCLC demonstrated a small benefit when it was used in combination with chemotherapy (median OS of 11.3) compared with chemotherapy alone (10.1 months, $P=0.044$) [27]. However, the median PFS was 4.8 months in both groups.

The most common AEs in this study were gastrointestinal and skin toxicities, which were generally mild and manageable. No deaths due to adverse events were reported. Some SAEs were reported including grade 2 cancer pain, which was attributable to the primary disease and was unrelated to either patritumab or erlotinib treatment. No DLTs were reported at either dosage levels (patritumab 9 or 18 mg/kg with oral daily dose of erlotinib 150 mg) and the doses tested did not reach MTD. Although most AEs in this study were similar to the well-known side effects of erlotinib, patritumab might have caused an incremental increase in the incidence of diarrhea compared with the incidence in a previous Japanese phase II study of erlotinib alone (95.8% *vs.* 74%) [28]. The incidence and rates of other grade 3 or 4 AEs including skin toxicities were similar to those in a previous erlotinib study [29]. Therefore, patritumab at a dose of 18 mg/kg in combination with an oral daily dose of erlotinib 150 mg was determined as the recommended dose for future studies in Japanese patients with NSCLC. The levels of pharmacokinetic parameters or patritumab in this study were similar to those observed in previous phase I studies of patritumab [18,19]. Furthermore, no neutralizing antibodies were detected in patients in this study after patritumab administration, as assessed by an anti-patritumab antibody and cell-based bioassay, similar to findings in previous studies [18,19].

In terms of efficacy of the combined treatment, 1 PR and 14 cases with SD were observed. The ORR was 4.2% and the DCR was 62.5%. The PR patient had a tumor with an *EGFR*-activating mutation and did not receive prior gefitinib treatment. The low ORR might be explained by the patient characteristics including the presence of wild-type *EGFR* and prior gefitinib treatment.

For the exploratory analysis, we separately evaluated the efficacy of this combination in patients with wild-type *EGFR* and those with *EGFR* mutations who developed resistance to gefitinib treatment. Of 9 patients with wild-type *EGFR*, 3 had SD (DCR 33%) and the median PFS for all 9 patients was 44.0 days. These results were not encouraging because they were similar to those obtained with the use of erlotinib alone in recent phase III studies in patients with previously treated NSCLC and wild-type *EGFR* (DCR 26% with a median PFS of 2.4 months [30], and DCR 52.8% with a median PFS of 1.3 months [31]). However, 10 SDs were observed in 12 patients with *EGFR* mutations who received prior gefitinib treatment. In the current study, the cohort DCR was 83.3% and the median PFS was 107 days. Although the results were limited because of the small number of patients, the DCR and median PFS are encouraging and suggest that patritumab might enhance the activity of erlotinib in patients with *EGFR*-activating mutations who develop resistance to gefitinib treatment, because the DCR and median PFS in a previous phase II study of erlotinib alone in patients after failure of gefitinib were only 28.6% and 60 days, respectively [32].

With respect to biomarkers, we investigated the correlation between HER3 expression in tumor tissues and the efficacy of combined patritumab and erlotinib treatment. Although recent studies suggested that HER3 was involved in the resistance to other HER receptor-targeted therapies [12], we observed no correlation between tumor response and HER3 expression in tumor tissues or serum soluble HER3 levels before treatment. An explanation for the lack of correlation between HER3 and efficacy might be the type of tumor tissues used in this study or the relatively low numbers of patients studied. Because we tested HER3 expression from tissue archived at the initial diagnosis, we could not examine HER3 expression just prior to this study. To confirm that hypothesis, it would be necessary to examine these biomarkers in larger numbers of patients in future studies.

In addition, serum soluble HER3 levels were significantly increased in all patients during treatment and serum soluble biomarkers were similar to those observed in previous studies of patritumab [19]. The mechanisms underlying these phenomena are unclear and require further study.

In conclusion, patritumab in combination with erlotinib was well tolerated up to 18 mg/kg without DLTs in previously treated Japanese NSCLC patients. This preliminary demonstration of the efficacy of the combined treatment was encouraging, especially in NSCLC patients with *EGFR*-activating mutations where prior gefitinib treatment failed.

Conflicts of interest

M. Nagashima and M. Sekiguchi are employed by Daiichi Sankyo. No potential conflicts of interest are disclosed by the other authors.

Role of the funding source

This study was funded by Daiichi Sankyo. The study sponsor contributed to the design of the study, was involved in the collection, analysis and interpretation of data, in the writing of the manuscript, and in the decision to submit the manuscript for publication.

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Pooled safety analysis of EGFR-TKI treatment for *EGFR* mutation-positive non-small cell lung cancer

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ABSTRACT

Objectives: Three epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKIs) – afatinib, erlotinib, and gefitinib – are available for the treatment of patients with *EGFR* mutation-positive non-small cell lung cancer (NSCLC). Given the long-term exposure of such patients to EGFR-TKIs, the toxicological properties of these agents in these individuals may differ from those observed in unselected patients. We compared the frequencies of severe adverse events (AEs) among *EGFR* mutation-positive NSCLC patients treated with these three EGFR-TKIs.

Materials and methods: We performed a pooled analysis of severe AEs according to the type of EGFR-TKI administered with the use of data extracted from prospective clinical trials that evaluated the clinical efficacy of gefitinib, erlotinib, or afatinib in NSCLC patients with *EGFR* mutations.

Results: Twenty-one trials published between 2006 and 2014 and including 1468 patients were eligible for analysis. Patients in 13 trials ($n = 457$) received gefitinib, those in 5 trials ($n = 513$) received erlotinib, and those in 3 trials ($n = 498$) received afatinib. Rash and diarrhea of grade ≥3 were significantly more frequent with afatinib therapy than with erlotinib or gefitinib therapy. The frequency of interstitial lung disease (ILD) of grade ≥3 was low (0.6–2.2%) with all three EGFR-TKIs and did not differ significantly among them. Gefitinib was associated with a significantly higher frequency of hepatotoxicity of grade ≥3 compared with erlotinib or afatinib. The overall frequency of AEs leading to treatment withdrawal was 6.1% (83 of 1354 evaluable patients), with such AEs occurring significantly more often with afatinib or gefitinib than with erlotinib. The most common withdrawal AEs were skin toxicity, ILD, and hepatotoxicity.

Conclusion: Such information on AEs should facilitate selection of the most appropriate EGFR-TKI for *EGFR* mutation-positive NSCLC patients with regard to mitigation of the risk for certain types of toxicity.

1. Introduction

Targeted therapies are under active development as a means to improve treatment efficacy in selected patient populations. Small-molecule tyrosine kinase inhibitors (TKIs) that target the epidermal growth factor receptor (EGFR), including the reversible inhibitors gefitinib and erlotinib, were the first targeted drugs to enter clinical use for the treatment of unselected patients with non-small cell lung cancer (NSCLC). Somatic mutations in the EGFR gene (*EGFR*) are associated with the therapeutic response to EGFR-TKIs in individuals with advanced NSCLC [1,2]. Indeed, randomized phase III studies revealed that first-line EGFR-TKI treatment resulted in an improved progression-free survival compared with standard chemotherapy in patients with advanced NSCLC who were selected on the basis of the presence of *EGFR* mutations [3–6]. Afatinib is an orally available drug that binds irreversibly to members of the ErbB family of proteins and thereby blocks signaling from EGFR (ErbB1), human epidermal growth factor receptor 2 (HER2, or ErbB2), ErbB4, and other relevant ErbB family dimers. The pivotal phase III studies, LUX-Lung 3 and 6 trials, showed that afatinib was superior to platinum doublets for the treatment of *EGFR* mutation-positive NSCLC [7,8]. The United States, European Union, and Japan recently approved afatinib for the treatment of NSCLC patients harboring *EGFR* mutations. Three EGFR-TKIs (afatinib, erlotinib, and gefitinib) are thus now available for first-line treatment of patients with *EGFR* mutation-positive NSCLC. Given the possible long-term exposure of *EGFR* mutation-positive patients to EGFR-TKIs, the toxicological properties of these drugs in such individuals may differ from those observed in unselected patients including those with or

Abbreviations: TKI, tyrosine kinase inhibitor; EGFR, epidermal growth factor receptor; NSCLC, non-small cell lung cancer; AE, adverse event; ILD, interstitial lung disease; OR, odds ratio; CI, confidence interval; TRD, treatment-related death.

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without *EGFR* mutations. Moreover, there have been no fully published clinical trials that have prospectively evaluated the effects of one EGFR-TKI in comparison with another in *EGFR* mutation-positive NSCLC patients. We have therefore now performed a pooled analysis of the occurrence of severe (grade ≥3) toxicity according to type of EGFR-TKI based on data extracted from phase II or III trials with advanced NSCLC patients positive for *EGFR* mutations.

2. Materials and methods

2.1. Search method

Trials were identified from a previous meta-analysis [9], a previous pooled analysis [10], and systematic computerized searches of the PubMed database encompassing the period from 1 June 2004 to 31 July 2014 with the key words "gefitinib," "erlotinib," "afatinib," "non-small cell lung cancer," "clinical trial," and "EGFR mutation." We chose to start with 2004 because this year marked the identification of *EGFR* mutations in NSCLC and their association with the response to EGFR-TKI treatment [1,2]. The included studies also had to (i) prospectively evaluate the clinical efficacy of gefitinib, erlotinib, or afatinib in NSCLC patients harboring *EGFR* mutations; (ii) present sufficient data on treatment safety including adverse events (AEs); and (iii) be full-text articles in English. When information about AEs leading to patient withdrawal was not available, we defined all AEs as nonwithdrawal toxicities if the trial described all treatment-related toxicities as "acceptable." We excluded studies that evaluated the combination of EGFR-TKIs with radiotherapy or experimental agents; case reports, letters, commentaries, or reviews; and abstracts of conference proceedings such as those of the American Society of Clinical Oncology and European Society for Medical Oncology.

2.2. Data extraction

The following data were extracted from all eligible studies: first author's name, year of publication, number of patients evaluable for toxicity, type of EGFR-TKI (gefitinib, erlotinib, or afatinib) administered, patient ethnicity, and number of patients experiencing toxicities (hepatotoxicity, rash, diarrhea, or interstitial lung disease) of grade ≥3. Afatinib studies were excluded from the ethnicity analysis because there was no afatinib trial that included only non-Asian patients [7,8,11]. Two authors (M.T. and I.O.) selected studies independently according to the aforementioned inclusion criteria. Safety data were collected for patients receiving erlotinib at 150 mg/day or gefitinib at 250 mg/day. With regard to afatinib, we used the toxicity data for patients receiving the U.S. Food and Drug Administration (FDA)-approved dose of 40 mg/day, although afatinib was given at a dose of 50 mg daily or, in a subsequent cohort, 40 mg daily in EGFR-TKI-naïve patients positive for *EGFR* mutations in one study [11]. AEs were reported on the basis of the National Cancer Institute Common Terminology Criteria (NCI-CTC) guidelines. Hepatotoxicity grade was defined on the basis of the higher value for either alanine aminotransferase (ALT) or aspartate aminotransferase (AST). Interstitial lung disease (ILD) that could be managed by interruption of EGFR-TKI treatment and by corticosteroid administration and was followed by resumption of EGFR-TKI treatment was defined as grade 2 [12]. Treatment-related death and toxicities that required temporary interruption of treatment were not included as withdrawal toxicities. ILD of grade 4 reported in one case was defined as a withdrawal toxicity [13]. The frequencies of AEs were compared among EGFR-TKIs with the use of Fisher's exact test. All *P* values were based on a two-tailed statistical analysis, and those of <0.05 were considered statistically significant. All statistical analysis was performed with GraphPad Prism software (version 5.0; GraphPad Software, San Diego, CA).

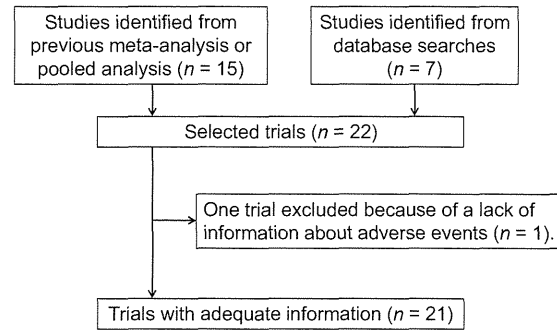

Fig. 1. Flowchart for study selection.

3. Results

3.1. Selected trials

On the basis of our search criteria, we identified 22 phase II or III trials of EGFR-TKIs for the treatment of patients with advanced NSCLC positive for *EGFR* mutations (Fig. 1). One of these trials was subsequently excluded because of a lack of information about AEs [14]. The remaining 21 trials [3–8,11–13,15–26], including a total of 1468 *EGFR* mutation-positive patients and published between 2006 and 2014, were eligible for the present study (Table 1). The sample size of the eligible trials ranged from 16 to 239. The patients of 13 studies (457 patients) received gefitinib, those of 5 studies (513 patients) received erlotinib, and those of 3 studies (498 patients) received afatinib.

3.2. Frequency of AEs of grade ≥3 according to type of EGFR-TKI

The frequency of hepatotoxicity of grade ≥3 was significantly greater in patients treated with gefitinib than in those treated with erlotinib [18% vs. 5.4%; odds ratio (OR) of 3.71 with a 95% confidence interval (CI) of 2.12–6.49; $P<0.0001$], as well as in those treated with erlotinib than in those treated with afatinib (5.4% vs. 1.7%; OR of 3.36 with a 95% CI of 1.11–10.2; $P=0.037$) (Fig. 2A).

Rash of grade ≥3 was significantly more frequent for afatinib than for erlotinib (15% vs. 8.8%; OR of 1.82 with a 95% CI of 1.23–2.69; $P=0.003$), as well as for erlotinib than for gefitinib (8.8% vs. 3.5%; OR of 2.65 with a 95% CI of 1.48–4.76; $P=0.0008$) (Fig. 2B).

Diarrhea of grade ≥3 also occurred significantly more often in the afatinib cohort than in the erlotinib cohort (9.6% vs. 2.7%; OR of 3.80 with a 95% CI of 2.07–6.99; $P<0.0001$), whereas the frequency of this toxicity did not differ significantly between erlotinib and gefitinib (2.7% vs. 1.1%; OR of 2.54 with a 95% CI of 0.91–7.10; $P=0.10$) (Fig. 2C).

Similar trends were observed for frequencies of toxicities of grade ≥2, in particular for hepatotoxicity and rash between gefitinib and erlotinib as well as for diarrhea between afatinib and erlotinib (Supplemental Table S1).

We also assessed the frequency of ILD of grade ≥3, because although this toxicity is rare it is potentially fatal. The frequency of ILD of grade ≥3 was low for all three EGFR-TKIs (2.2% for gefitinib, 0.6% for erlotinib or afatinib) and did not differ significantly among the three cohorts (gefitinib vs. erlotinib, OR of 3.76 with a 95% CI of 0.99–14.3 and $P=0.06$; afatinib vs. erlotinib, OR of 1.10 with a 95% CI of 0.22–5.46 and $P=1.0$) (Fig. 2D). A similar pattern was observed for the frequency of ILD of grade ≥2 (Supplemental Table S1).

Table 1
Characteristics of the eligible trials.

First author	Year	Treatment	Ethnicity	No. of patients	No. of toxicities of grade ≥3			
					Hepatotoxicity	Rash	Diarrhea	ILD
Asahina [15]	2006	Gefitinib	Asian	16	2	1	0	0
Inoue [16]	2006	Gefitinib	Asian	16	1	0	0	0
Sutani [17]	2006	Gefitinib	Asian	27	1	1	0	0
Sunaga [18]	2007	Gefitinib	Asian	21	1	1	0	1
Yoshida [19]	2007	Gefitinib	Asian	21	3	0	2	0
Tamura [20]	2008	Gefitinib	Asian	28	5	2	0	1
Sequist [21]	2008	Gefitinib	Non-Asian	31	1	0	0	1
Sugio [22]	2009	Gefitinib	Asian	19	0	0	0	0
Rosell [12]	2009	Erlotinib	Non-Asian	217	NA	16	8	0
Inoue [13]	2009	Gefitinib	Asian	29	3	0	0	1
Mitsudomi [3]	2010	Gefitinib	Asian	87	24	2	1	NA
Maemondo [4]	2010	Gefitinib	Asian	114	30	6	1	3
Asami [23]	2011	Gefitinib	Asian	17	3	2	0	0
Zhou [5]	2011	Erlotinib	Asian	83	3	2	1	0
Rosell [6]	2012	Erlotinib	Non-Asian	84	3	11	4	1
Maemondo [24]	2012	Gefitinib	Asian	31	6	1	1	1
Yang [11]	2012	Afatinib	Multiple	30	NA	2	2	NA
Yamada [25]	2013	Erlotinib	Asian	26	2	2	0	0
Goto [26]	2013	Erlotinib	Asian	103	8	14	1	2
Sequist [7]	2013	Afatinib	Multiple	229	NA	37	33	2
Wu [8]	2014	Afatinib	Asian	239	4	35	13	1

NA, not applicable.

To investigate whether the line of treatment might affect the incidence of toxicities of grade ≥3, we analyzed the frequency of such toxicities in patients receiving EGFR-TKIs in the first-line setting (Supplemental Fig. S1). The differences in toxicity frequencies among the three EGFR-TKIs in this setting (13 studies) were similar to those apparent in the analysis of all 21 trials included in our study.

3.3. Frequency of AEs of grade ≥3 according to patient ethnicity

The frequency of gefitinib-related hepatotoxicity of grade ≥3 was significantly higher in Asians than in non-Asians (18.5% vs. 3.2%; OR of 6.83 with a 95% CI of 0.92–50.9; $P=0.027$), whereas the frequency of erlotinib-related hepatotoxicity of grade ≥3 did not differ significantly between these two ethnic groups (6.1% vs. 3.6%; OR of 1.76 with a 95% CI of 0.49–6.36; $P=0.57$) (Fig. 3A).

The frequency of rash of grade ≥3 did not differ significantly between Asians and non-Asians treated either with gefitinib (3.8% vs. 0.0%; OR of 2.53 with a 95% CI of 0.15–43.2; $P=0.62$) or with erlotinib (8.5% vs. 9.0%; OR of 0.94 with a 95% CI of 0.50–1.76; $P=0.88$) (Fig. 3B).

There was also no significant difference in the frequency of diarrhea of grade ≥3 between Asians and non-Asians treated either with gefitinib (1.2% vs. 0.0%; OR of 0.82 with a 95% CI of 0.04–15.2; $P=1.0$) or with erlotinib (0.9% vs. 4.0%; OR of 0.23 with a 95% CI of 0.05–1.04; $P=0.05$) (Fig. 3C).

Similarly, the frequency of ILD of grade ≥3 did not differ significantly between Asians and non-Asians treated either with gefitinib (2.1% vs. 3.2%; OR of 0.63 with a 95% CI of 0.08–5.32; $P=0.51$) or with erlotinib (0.9% vs. 0.3%; OR of 2.86 with a 95% CI of 0.26–31.7; $P=0.57$) (Fig. 3D). The frequency of all-grade ILD in Asian and non-Asian patients was 2.5% and 0.9%, respectively (OR of 2.79 with a 95% CI of 0.82–9.40; $P=0.11$). In particular, that in Japanese and non-Japanese Asian patients was 3.8% and 0.3%, respectively (OR of 12.7 with a 95% CI of 1.69–95.1; $P=0.0009$).

3.4. Frequency of treatment-related death according to type of EGFR-TKI

Treatment-related death (TRD) was reported in 12 of the 1438 evaluable patients, giving an overall prevalence of 0.8%. The main cause of such death was ILD (7 of 12 patients). Four of 457 patients (0.9%) experienced TRD (due to ILD) in the gefitinib group, 3 of 513 patients (0.6%) experienced TRD (due to hepatotoxicity in 1 patient and ILD in 2 patients) in the erlotinib group, and 5 of 468 patients (1.1%) experienced TRD (due to dyspnea, ILD, and sepsis in one patient each, and to unknown causes in two cases) in the afatinib group.

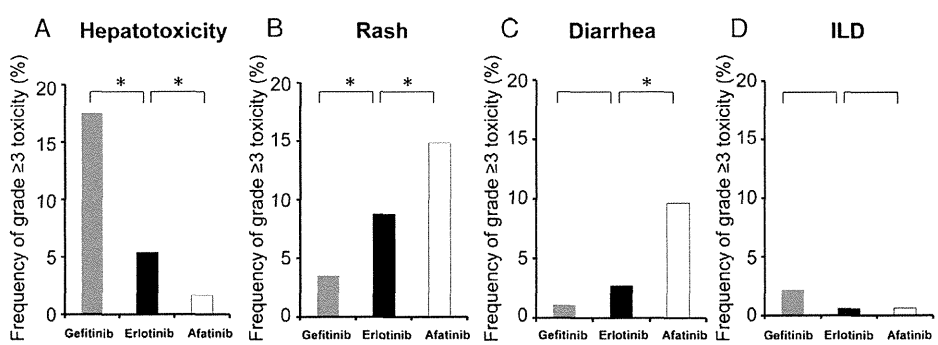

Fig. 2. Frequency of AEs of grade ≥3 including hepatotoxicity (A), rash (B), diarrhea (C), and ILD (D) according to type of EGFR-TKI. Asterisks indicate statistically significant differences.

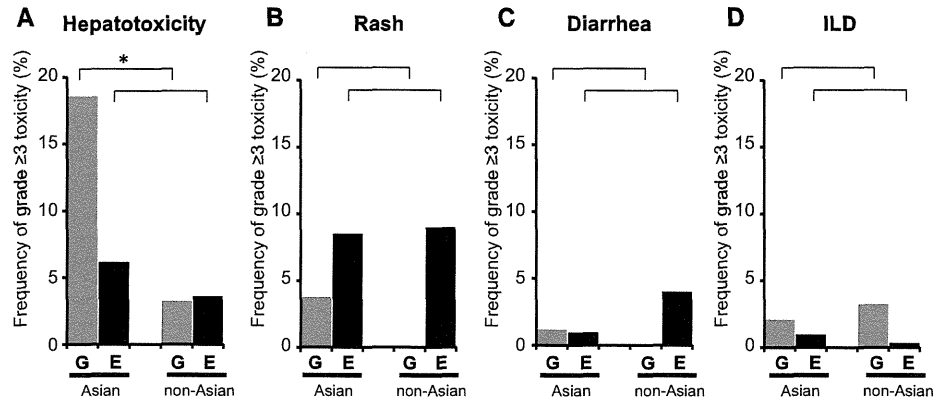

Fig. 3. Frequency of AEs of grade ≥3 including hepatotoxicity (A), rash (B), diarrhea (C), and ILD (D) according to ethnic background and type of EGFR-TKI. G, gefitinib; E, erlotinib. Asterisks indicate statistically significant differences.

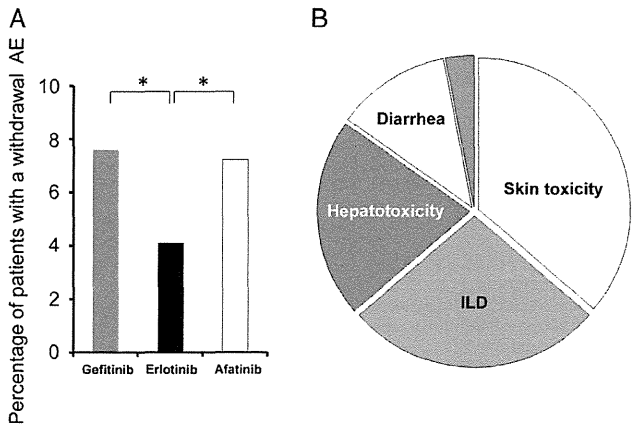

Fig. 4. Frequency of withdrawal AEs according to type of EGFR-TKI (A) and AEs responsible for discontinuation of EGFR-TKI treatment (B). Asterisks indicate statistically significant differences.

3.5. *Identification of withdrawal toxicities according to type of EGFR-TKI*

A subset of NSCLC patients with *EGFR* mutations discontinues EGFR-TKI treatment as a result of AEs. The overall frequency of AEs leading to treatment withdrawal was 6.1% (83 of 1354 evaluable patients). Such withdrawal AEs were significantly more frequent in the afatinib group than in the erlotinib group (7.2% vs. 4.1%, $P=0.040$), as well as in the gefitinib group than in the erlotinib group (7.6% vs. 4.1%, $P=0.032$) (Fig. 4A). Of the 83 patients in whom EGFR-TKI treatment was discontinued because of AEs, the reason for treatment withdrawal was available for 33 patients (12, 10, and 11 patients treated with gefitinib, erlotinib, and afatinib, respectively) (Fig. 4B). The most common withdrawal AEs were related to skin toxicity (3, 2, and 7 patients treated with gefitinib, erlotinib, and afatinib, respectively), followed by ILD (4, 4, and 1 patients treated with gefitinib, erlotinib, and afatinib, respectively), hepatotoxicity (4 and 3 patients treated with gefitinib and erlotinib, respectively), diarrhea (1 and 3 patients treated with gefitinib and afatinib, respectively), and spleen cyst (1 patient treated with erlotinib).

4. Discussion

Given the long-term exposure of *EGFR* mutation-positive patients to EGFR-TKIs, the toxicological properties of these agents in such patients may differ from those observed in unselected patients. The INTEREST phase III trial [27], which enrolled unselected patients and randomly assigned them to receive gefitinib or docetaxel, as well as the ZEST phase III trial [28], which compared vandetanib with erlotinib also in unselected patients, found that all-grade hepatotoxicity related to gefitinib or erlotinib occurred in <10% of treated individuals. In contrast, our pooled analysis has revealed that the frequency of all-grade hepatotoxicity induced by gefitinib or erlotinib was 48% and 27%, respectively (data not shown), whereas the frequency of hepatotoxicity of grade ≥3 was 18% and 5.4%, respectively. These results indicate that long-term exposure to gefitinib or erlotinib is associated with an increased frequency of hepatotoxicity. The reason for the difference in the frequency of gefitinib-related hepatotoxicty of grade ≥3 between Asian and non-Asian patients in our analysis is unclear. CYP2D6 contributes to the metabolism of gefitinib but not to that of erlotinib [29]. The frequency of nonfunctional alleles of the CYP2D6 gene is greater in Asians than in Caucasians [30], suggesting that differences in drug pharmacokinetics may affect the development of hepatotoxicity. Exacerbation of preexisting liver disease, especially viral hepatitis, also cannot be excluded as a possible mechanism leading to hepatotoxicity, given that the prevalence of hepatitis B antigen carriage is low (0.1–2.0%) in the United States and Northern and Western Europe, whereas it is intermediate (2.0–8.0%) in Japan and high (8.0–20.0%) in Southeast Asia and China [31]. Patients who are infected with hepatitis B virus may thus be at increased risk for gefitinib-related hepatotoxicity.

Our pooled analysis has shown that rash or diarrhea of grade ≥3 occurs significantly more often among patients treated with afatinib than among those treated with erlotinib or gefitinib. These differences in the frequency of rash and diarrhea may be related to the mechanism of drug action. EGFR is expressed in epithelia and helps to maintain mucosal integrity and to promote mucosal repair in the gut as well as to maintain the protective barrier of the skin. Afatinib has a higher affinity for the kinase domain of EGFR than does gefitinib or erlotinib, and the irreversible tyrosine kinase blockade mediated by afatinib may result in more sustained suppression of ErbB signaling compared with that induced by reversible inhibitors such as gefitinib and erlotinib [32].

Three large Japanese studies of ILD associated with EGFR-TKI treatment in unselected patients found that the frequency of gefitinib-related ILD is 3.5 to 4.0% and that of erlotinib-related ILD is 4.5% [33–35]. On the other hand, the prevalence of ILD in unselected patients treated with gefitinib was 0.3% in a U.S. AstraZeneca Expanded Access Program [36]. These data suggest that ethnic differences may affect the clinical manifestation of ILD in unselected patients treated with EGFR-TKIs. Our pooled analysis of EGFR-TKI

treatment in *EGFR* mutation-positive NSCLC patients found that the frequency of all-grade ILD in Asian and non-Asian studies was 2.5% and 0.9%, respectively. In particular, that in Japanese and non-Japanese Asian patients was 3.8% and 0.3%, respectively. The reason for this difference in the frequency of EGFR-TKI-related ILD between Japan and other countries remains unclear. It is possible that a greater awareness of the disease in Japan leads to more careful and critical examination of patients for this condition, or that Japanese have an increased genetic susceptibility to ILD.

A small proportion of NSCLC patients with *EGFR* mutations discontinues EGFR-TKI treatment as a result of AEs. Our pooled analysis has revealed that the frequency of total AEs leading to withdrawal was 6.1%, with the most common such AEs being those related to skin toxicity, ILD, and hepatotoxicity. This withdrawal profile may not be representative, however, given that the criteria for discontinuation of EGFR-TKI treatment varies among protocols. The effective management of EGFR-TKI-related AEs has improved in more recent years of our study period, with the result that the proportion of patients required to discontinue EGFR-TKI treatment according to protocol may differ between more recent and earlier studies.

In conclusion, whereas treatment with EGFR-TKIs has become established for patients with advanced NSCLC positive for activating *EGFR* mutations, we have now shown that severe rash and diarrhea occur significantly more frequently in such patients treated with afatinib than in those treated with erlotinib or gefitinib, and that treatment with gefitinib is associated with an increased frequency of severe hepatotoxicity. Such information on the frequency of severe AEs according to the type of EGFR-TKI administered should help in selection of the most appropriate EGFR-TKI for *EGFR* mutation-positive NSCLC patients with regard to mitigation of the risk for certain types of toxicity.

Conflict of interest statement

I.O. and K.N. have received honoraria from Chugai Pharmaceuticals, AstraZeneca, and Boehringer Ingelheim.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.lungcan.2015.01.026.

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Chronic nicotine exposure mediates resistance to EGFR-TKI in *EGFR*-mutated lung cancer *via* an EGFR signal

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ABSTRACT

Background: Some of patients with non-small cell lung cancer (NSCLC) harboring somatic activating mutations of the epidermal growth factor receptor gene (*EGFR* mutations) show poor responses to EGFR-tyrosine kinase inhibitors (EGFR-TKIs) treatment. Cigarette smoking is the strongest documented risk factor for the development of lung cancer. Nicotine, while not carcinogenic by itself, has been shown to induce proliferation, angiogenesis, and the epithelial-mesenchymal transition; these effects might be associated with EGFR-TKI resistance.

Materials and methods: PC-9 and 11_18 cell lines (*EGFR*-mutated NSCLC cell lines) were cultured with 1 μM nicotine for 3 months and were designated as PC-9/N and 11_18/N cell lines, respectively. The sensitivities of these cell lines to EGFR-TKI were then tested *in vitro*. Moreover, the association between the smoking status and the progression-free survival (PFS) period was investigated in patients with *EGFR*-mutated NSCLC who were treated with gefitinib.

Results: The PC-9/N and 11_18/N cell lines were resistant to EGFR-TKI, compared with controls. The phosphorylation of EGFR in these cell lines was reduced by EGFR-TKI to a smaller extent than that observed in controls, and a higher concentration of EGFR-TKI was capable of further decreasing the phosphorylation. Clinically, smoking history was an independent predictor of a poor PFS period on gefitinib treatment.

Conclusions: Chronic nicotine exposure because of cigarette smoking mediates resistance to EGFR-TKI *via* an EGFR signal. Smoking cessation is of great importance, while resistance may be overcome through the administration of high-dose EGFR-TKI.

1. Introduction

Lung cancer is the leading cause of cancer-related death in the developed world [1]. Non-small cell lung cancer (NSCLC) accounts for approximately 80% of lung cancers, and the prognosis of advanced NSCLC remains very poor despite advances in treatment [2].

Epidermal growth factor receptor (EGFR) is recognized as an important molecular target in cancer therapy [3], and the somatic activating mutation of the EGFR gene (*EGFR* mutation) is an oncogenic driver mutation in NSCLC [4]. Patients with NSCLC harboring *EGFR* mutations generally respond to EGFR-tyrosine kinase inhibitors (EGFR-TKIs) [5–10]. Although these patients respond dramatically to such treatment, some of them show poor response and the majority eventually undergo disease progression [11]. Many studies have revealed several resistance mechanisms and candidates, including *EGFR* secondary mutations, MET gene amplification, and epithelial-mesenchymal transition (EMT) [12–14].

Cigarette smoking is the strongest documented risk factor for the development of lung cancer. Tobacco-derived carcinogens are known to form DNA adducts, mutate vital growth regulatory genes such as p53 and Ras, and initiate oncogenesis [15,16]. Nicotine, while not carcinogenic by itself, has been shown to induce proliferation, angiogenesis, and EMT in several experimental models [17–19]. Previous preclinical studies have shown that cigarette smoke exposure renders EGFR-TKIs ineffective in *EGFR*-mutated cell lines [20,21]. In addition, another study has demonstrated that short-term nicotine exposure activates an EGFR signal and induces

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