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Interstitial Lung Disease in Japanese Patients with Lung Cancer

A Cohort and Nested Case-Control Study

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Rationale: **Interstitial lung disease (ILD) occurs in Japanese patients with non–small cell lung cancer (NSCLC) receiving gefitinib.**

Objectives: **To elucidate risk factors for ILD in Japanese patients with NSCLC during treatment with gefitinib or chemotherapy.**

Methods: **In a prospective epidemiologic cohort, 3,166 Japanese patients with advanced/recurrent NSCLC were followed for 12 weeks on 250 mg gefitinib (n = 1,872 treatment periods) or chemotherapy (n = 2,551). Patients who developed acute ILD (n = 122) and randomly selected control subjects (n = 574) entered a case-control study. Adjusted incidence rate ratios were estimated from case-control data by odds ratios (ORs) with 95% confidence intervals (CIs) using logistic regression. Crude (observed) incidence rates and risks were calculated from cohort data.**

Measurements and Main Results: **The observed (unadjusted) incidence rate over 12 weeks was 2.8 (95% CI, 2.3–3.3) per 1,000 person-weeks, 4.5 (3.5–5.4) for gefitinib versus 1.7 (1.2–2.2) for chemotherapy; the corresponding observed naive cumulative incidence rates at the end of 12-week follow-up were 4.0% (3.0–5.1%) and 2.1% (1.5–2.9%), respectively. Adjusted for imbalances in risk factors between treatments, the overall OR for gefitinib versus chemotherapy was 3.2 (1.9–5.4), elevated chiefly during the first 4 weeks (3.8 [1.9–7.7]). Other ILD risk factors in both groups included the following: older age, poor World Health Organization performance status, smoking, recent NSCLC diagnosis, reduced normal lung on computed tomography scan, preexisting chronic ILD, concurrent cardiac disease. ILD-related deaths in patients with ILD were 31.6% (gefitinib) versus 27.9% (chemotherapy); adjusted OR, 1.05 (95% CI, 0.3–3.2).**

Conclusions: **ILD was relatively common in these Japanese patients with NSCLC during therapy with gefitinib or chemotherapy, being higher in the older, smoking patient with preexisting ILD or poor performance status. The risk of developing ILD was higher with gefitinib than chemotherapy, mainly in the first 4 weeks.**

Keywords: non–small cell lung cancer; interstitial lung disease; Japanese patients; gefitinib, chemotherapy

AT A GLANCE COMMENTARY

Scientific Knowledge on the Subject

Acute interstitial lung disease (ILD) occurs in Japanese patients with non–small cell lung cancer (NSCLC) receiving gefitinib. There is, however, limited knowledge about risk factors for ILD and the incidence of ILD in patients with NSCLC receiving other treatments.

What This Study Adds to the Field

Acute ILD was common in Japanese patients with NSCLC receiving chemotherapy or gefitinib, with higher risk for gefitinib. Age, performance status, smoking, and preexisting chronic ILD were also important risk factors, aiding clinicians in treatment selection.

Epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors are a well-established therapy for the treatment of non–small cell lung cancer (NSCLC) in many countries. They are generally well tolerated and not typically associated with the cytotoxic side effects commonly seen with chemotherapy.

The EGFR tyrosine kinase inhibitor gefitinib (IRESSA; AstraZeneca, London, U.K.) was first approved for the treatment of advanced NSCLC in Japan in July 2002. In clinical trials and in preapproval compassionate clinical use, some reports of interstitial lung disease (ILD)–type events had been observed. As the drug was made more widely available in Japan after approval, however, an increasing number of spontaneous reports for ILD appeared.

ILD is a disease that affects the parenchyma or alveolar region of the lungs (1). When associated with drug use, it can present precipitously with acute diffuse alveolar damage, which is fatal in some patients (2). Chest imaging shows ground-glass density and patients present with severe breathlessness. There is no specific treatment, but supportive therapy including oxygen, corticosteroids, or assisted ventilation is indicated. Acute exacerbations of ILD have previously been considered relatively rare in many settings, with Japan as a notable exception (3), but recent studies of patients with idiopathic pulmonary fibrosis (IPF) have challenged this and underlined this important risk (4).

ILD, especially IPF, is a known comorbidity in patients with NSCLC and has also been associated with many other lung cancer therapies (5). Rates of acute ILD events up to and exceeding 10% have been reported in patients receiving chemotherapy and radiotherapy (6–11). It is recognized that ILD is more common in Japan than elsewhere (5, 6, 12, 13).

(Received in original form October 11, 2007; accepted in final form March 12, 2008)

Supported by AstraZeneca.

*A list of Japan Thoracic Radiology Group members may be found in the ACKNOWLEDGMENT.

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This article has an online supplement, which is accessible from this issue's table of contents at www.atsjournals.org

Am J Respir Crit Care Med Vol 177. pp 1348–1357, 2008
Originally Published in Press as DOI: 10.1164/rccm.200710-1501OC on March 12, 2008
Internet address: www.atsjournals.org

When safety reports of acute ILD-type events in gefitinib-treated patients appeared in Japan, there was limited knowledge about ILD in patients with NSCLC. There was a need to better understand baseline incidence on different treatments, risk factors for developing ILD, and whether gefitinib might be associated with increased risk of ILD, or if patient selection or other aspects were involved. A pharmacoepidemiologic study was designed and conducted by an independent academic team together with scientists from AstraZeneca to define the risk and increase understanding of ILD in Japanese patients with NSCLC. Some of the results of this study have been previously reported in the form of conference abstracts (14, 15).

METHODS

See also the online supplement for further details on methods.

Overall Study Design

A nonrandomized cohort study with a nested case-control study component was conducted between November 12, 2003, and February 22, 2006, in 50 centers across Japan. Patients with advanced or recurring NSCLC who had received at least one chemotherapy regimen were eligible for cohort entry. Patients and their physicians selected the most appropriate treatment (gefitinib 250 mg or chemotherapy) and the patients were followed for up to 12 weeks after treatment initiation. Basic data were collected at the start of follow-up and included sex, age, World Health Organization (WHO) performance status (PS), and tumor histology. If a patient switched to a new treatment, he or she could be re-enrolled for a new treatment period of 12 weeks, provided he or she was still eligible.

Patients who developed acute ILD events during the follow-up were registered to the case-control study nested within the cohort as clinically diagnosed potential cases. For each potential case, four patients who had not yet developed ILD were randomly selected as appropriate control subjects from patients registered to the cohort at that time, and extensive clinical and demographic risk factor data were collected on cases and control subjects (*see* Figure E1 in the online supplement).

The study followed Good Clinical Practice procedures. An independent external epidemiology advisory board provided advice on design, conduct, and analysis of the study.

Diagnosis of ILD

To ensure an accurate diagnosis of ILD, several study design components were implemented: (*1*) an information card to all cohort patients, alerting them to the symptoms of ILD; (*2*) internationally agreed criteria for the diagnosis of ILD and a diagnostic algorithm (*see* Figure E2) developed from the American Thoracic Society/European Respiratory Society consensus statement (1); and (*3*) a blinded diagnostic review of all clinically diagnosed potential ILD cases registered to the study by an independent case review board (CRB) of radiologists and clinicians.

Evaluation of Preexisting Lung Conditions

The CRB also blindly evaluated pretreatment computed tomography (CT) scans for the presence of a number of pulmonary conditions: preexisting (chronic) ILD (mainly IPF), drug-induced lung disease, pulmonary emphysema, radiation pneumonitis, lymphangitis carcinomatosis, and healed tuberculosis, and evaluated the extent of normal lung, as well as the extent of areas adherent to pleura.

Detailed Data Collection

For cases and control subjects, detailed data on NSCLC treatment, demography, cancer histology, clinical stage and the presence of metastases, WHO PS, smoking, previous cancer treatments, past and current medical history, surgical history, and concomitant medication and therapy were collected. Data on serious adverse events (SAEs) and hence all-cause mortality were collected for the gefitinib-treated patients in the cohort only; thus, information on mortality from causes other than ILD in chemotherapy-treated patients is not available from this study.

Statistical Analysis

From cohort data, we estimated observed person-time incidence rates as well as two measures of the observed "risk" of acute ILD to a patient; a naive estimate of observed cumulative incidence (incidence proportion, "frequency"), and risk up to 84 days by the Kaplan-Meier method.

Control subjects for the nested case-control study were sampled using incidence density sampling, and consequently the odds ratio (OR) obtained from the case-control analysis estimates the study incidence rate ratio (and approximately estimates the risk ratio) (16).

For the case-control statistical analysis, it was initially verified that the convenience matching for calendar time implicit in the risk set control sampling could be disregarded. In tabular analyses, we then identified potential confounders and risk factors, using as selection criteria a 10% change in the OR estimate for gefitinib versus chemotherapy treatment when stratifying for each factor separately, and a risk factor crude OR of less than 0.5 or more than 2.0, respectively. We also identified potential interactions between treatment and other risk factors, or between two potential risk factors. Modeling using logistic regression then proceeded in the corresponding four steps. Few previous data were available on risk factors for ILD in patients with NSCLC and so a hypothesis-free stepwise process with loose P value criteria ($P < 0.20$) for selection was used throughout to avoid bias.

Two sensitivity analyses were performed. First, to investigate the potential influence of the modeling approach used, a propensity score analysis was performed (17). This analysis provides an alternative way of adjusting for potential confounding bias by stratifying for a compound score based on predictors of treatment (*see* online supplement for details). Second, we estimated the possible bias due to misclassification of disease under reasonable assumptions of diagnostic error.

ILD-related mortality among the patients who developed acute ILD on gefitinib or chemotherapy treatment was obtained. Modeling of risk factors for ILD-related mortality followed a similar process to the ILD risk factor modeling. For gefitinib-treated patients, two additional data items were available: total all-cause mortality, which was analyzed by the Kaplan-Meier method, and SAEs, for which frequencies and possible consequences in terms of treatment discontinuation and death were calculated.

RESULTS

Cohort Subjects and Treatments

Cohort participation rates were high. In 10 sampled study centers, 89.6% of eligible patients were enrolled to the cohort. The number of treatment periods and subjects are summarized in Table 1. In total, 4,423 treatment periods in 3,159 subjects were available for analysis. In the cohort, 70.8% of patients had only one treatment period, 21.5% had two periods, and the remaining 7.8% of patients had three or more treatment periods registered (Table 1). Chemotherapy included a wide range of treatments, the most common being taxane monotherapy, followed by taxane+platinum and gemcitabine+vinorelbine combinations.

Cases and Control Subjects

In the overall cohort data of all treatment periods, clinicians reported 155 suspected cases of acute ILD during the follow-up, of which 122 were confirmed by the CRB after blinded review of CT and clinical data—79 of 103 gefitinib-treated (76.7%) and 43 of 52 chemotherapy-treated (82.7%) subjects. A total of 574 eligible control subjects were sampled from the person-time of the cohort. Almost all ILD cases and selected control subjects consented to participate in the nested case-control study, with final participation rates of 98.1 and 92.0%, respectively. Valid data from the CRB review of CT scans were available for 115 cases and 520 control subjects.

Descriptive Data

On data items available for the full cohort (sex, age, WHO PS, and tumor histology), the control subjects were quite represen-

TABLE 1. NUMBER OF TREATMENT PERIODS AND SUBJECTS IN THE COHORT AND NUMBER OF CASES AND CONTROLS IN THE NESTED CASE-CONTROL STUDY

	Gefitinib (*n*)	Chemotherapy (*n*)	Total (*n*)
Treatment periods registered to cohort	1,901	2,572	4,473
No treatment administered	9	15	24
Ineligible subjects	6	6	12
Protocol deviations	14	0	14
Per-protocol study cohort (treatment periods)	1,872	2,551	4,423
Subjects in cohort (first treatment periods)*	1,489	1,677	3,166
No. of subjects and order of treatment periods registered to the cohort			
1 treatment period: G			1,199
1 treatment period: C			1,036
2 treatment periods: GC			194
2 treatment periods: CG			248
2 treatment periods: CC			228
2 treatment periods: GG			9
3–8 treatment periods†: initial G			81
3–9 treatment periods‡: initial C			166
First gefitinib treatment periods total§	1,849		
Confirmed cases‖	79	43	122
Rejected cases‖	24	9	33
Control subjects	252	322	574

Definition of abbreviations: C = chemotherapy; G = gefitinib.

* Counts the first registered treatment period for each subject.

† 70% of these with three periods.

‡ 78% of these with three periods.

§ Counts the first gefitinib treatment period for all subjects with one or more gefitinib treatment registrations to the cohort; also when their very first registration was for chemotherapy.

‖ Cases registered by clinical investigators to the case-control study and subsequently confirmed or rejected by the case review board (blinded review of case diagnostic data).

tative of the overall cohort (details not shown). Comparisons of the gefitinib- and chemotherapy-treated control groups as representative of the cohort indicated that the former included more women, never-smokers, adenocarcinoma tumors, and poorer PS, as well as less preexisting ILD and pulmonary emphysema on CT scan (Tables 2 and 3). ILD cases, regardless of treatment, were more likely than cohort control subjects to be older, male, smokers, with squamous cell carcinoma histology, and have poor PS (Tables 2 and 3). The frequency of preexisting ILD and pulmonary emphysema was higher in cases, reflected also in a lower extent of normal lung on CT scan.

Cohort Analysis of ILD Occurrence

The observed incidence rate of acute ILD over the entire 12-week follow-up in the overall cohort was 2.8 per 1,000 person-weeks—4.5 in the gefitinib-treated and 1.7 in the chemotherapy subcohort (Table 4). The observed incidence in the gefitinib-treated subcohort was highest in the first 4 weeks after starting treatment, greater than in the chemotherapy-treated subcohort. In the following two 4-week periods, the incidence was lower with no clear difference (Table 4, Figure 1A). The naive cumulative incidence of ILD at 84 days (i.e., observed frequency or proportion of the original cohort that developed ILD in the study) for patients in their first study treatment period was 4.0 and 2.1% for gefitinib- and chemotherapy-treated patients, respectively (Table 4), whereas the estimated theoretical 12-week risk of ILD (i.e., taking competing causes of death and loss to follow-up into consideration; Kaplan-Meier method) was 4.5 and 2.4%, respectively (Table 4, Figure 1B). Thus, the observed cohort rates and risks suggested an association of increased ILD occurrence with gefitinib treatment mainly in the first 4 weeks after treatment initiation. All cohort estimates are unadjusted for imbalances between treatments in other risk factors. Detailed comparisons between the treatments therefore used the adjusted case-control OR (as an estimate of the adjusted incidence rate ratio) to achieve comparability.

Case-Control Analysis of ILD Occurrence and Risk Factors

Major results. The OR of developing acute ILD with gefitinib treatment versus chemotherapy, adjusted for the full predictor model of major confounders together with additional identified important risk factors and interactions, was 3.2 (95% confidence interval [CI], 1.9–5.4) (Table 5). Several risk factors aside from treatment also had strong effects, including WHO PS, as well as smoking status and preexisting ILD together with the extent of normal lung on CT scan, which interacted in a complex way in the model (Table 5, Figure 2). Preexisting ILD was confirmed as a strong risk factor, with OR point estimates ranging from 4.8 to 25.3 depending on the extent of remaining normal lung on CT scan, in comparison with patients without preexisting ILD and high extent of normal lung on CT scan (Table 5). The full set of ILD risk factors in both groups from the final model thus included older age (≥55 yr), WHO PS (≥2), smoking, short duration since NSCLC diagnosis (<6 mo), reduced extent of normal lung on CT scan (<50%), preexisting ILD, and concurrent cardiac disease. Although some potential significant interactions were seen in the initial tabular analyses (Table E1), no significant interactions with treatment (i.e., treatment-specific risk factors, or variation in treatment-related effect in subgroups defined by another risk factor) were identified in the modeling after adjustment for the relevant risk factors.

When the case-control analyses focused on the first 4 weeks after treatment initiation (because the unadjusted cohort analyses above indicated that the bulk of the association with gefitinib appeared to be for this time interval) the estimated OR adjusted for a full predictor model developed on this period's data was 3.8 (95% CI, 1.9–7.7). The same model produced an OR for Weeks 5–8 of 1.6 (95% CI, 0.5–4.8), whereas the final 4-week period had too few cases for an adequate estimate. The estimate for Weeks 5–12 combined, using this same model, was 2.5 (95% CI, 1.1–5.8). The important covariates and predictors were the same in this model as in the model for the full 12-week data, with the exception of age, preexisting cardiac disease, and preexisting pulmonary emphysema, which were not included. Due to sparse data beyond 4 weeks, independent models for Weeks 5–8, 9–12, and 5–12 could not be developed.

Confounding and sensitivity analysis. In the overall 12-week basic analysis, moderately strong confounding by other risk factors was found. The crude OR of developing ILD with gefitinib treatment versus chemotherapy was 2.3 (95% CI, 1.5–3.6). When adjusted for some of the most important potential confounders one at a time, the adjusted OR point estimate for the association of treatment with ILD occurrence ranged from 2.1 to 3.1 (*see* Table E1 for details). The most important confounder was severity of preexisting ILD with strong negative confounding, and the only one that resulted in a lower adjusted OR than 2.3 (positive confounding) was WHO PS.

The propensity score analysis approach identified the following variables as the most important predictors of selecting gefitinib treatment in this study: female sex; nonsmoking status; non–squamous tumor histology; poor PS; preexisting lymphangitis carcinomatosis; no previous gefitinib treatment; and no preexisting ILD, emphysema, or radiation pneumonitis. The

TABLE 2. CHARACTERISTICS OF CONFIRMED CASES AND CONTROL SUBJECTS (AS A RANDOM SAMPLE OF THE STUDY COHORT)

	Cases (*n* = 122)	Controls (*n* = 574)	Gefitinib Control Sample (*n* = 252)	Chemotherapy Control Sample (*n* = 322)
Sex				
Male	92 (75.4)	360 (62.7)	126 (50.0)	234 (72.7)
Female	30 (24.6)	214 (37.3)	126 (50.0)	88 (27.3)
Age				
<55 yr	11 (9.0)	95 (16.6)	43 (17.1)	52 (16.1)
≥55 yr	111 (91.0)	479 (83.4)	209 (82.9)	270 (83.9)
WHO performance status				
0	18 (14.8)	154 (26.8)	68 (27.0)	86 (26.7)
1	69 (56.6)	358 (62.4)	148 (58.7)	210 (65.2)
2–3	35 (28.7)	62 (10.8)	36 (14.3)	26 (8.1)
Histologic type				
Squamous cell carcinoma	29 (23.8)	103 (17.9)	27 (10.7)	76 (23.6)
Adenocarcinoma	80 (65.6)	414 (72.1)	207 (82.1)	207 (64.3)
Others	13 (10.7)	57 (9.9)	18 (7.1)	39 (12.1)
Smoking history				
No	21 (17.2)	192 (33.4)	113 (44.8)	79 (24.5)
Yes	100 (82.0)	382 (66.6)	139 (55.2)	243 (75.5)
Unknown	1 (0.8)	0 (0.0)	0 (0.0)	0 (0.0)
Time since diagnosis of NSCLC				
<0.5 yr	49 (40.2)	153 (26.7)	65 (25.8)	88 (27.3)
0.5 to <1 yr	36 (29.5)	154 (26.8)	67 (26.6)	87 (27.0)
≥1 yr	37 (30.3)	267 (46.5)	120 (47.6)	147 (45.7)
Previous gefitinib treatment				
No	113 (92.6)	465 (81.0)	241 (95.6)	224 (69.6)
Yes	9 (7.4)	109 (19.0)	11 (4.4)	98 (30.4)
Concurrent cardiac disease				
No	111 (91.0)	556 (96.7)	244 (96.4)	312 (96.9)
Yes	11 (9.0)	19 (3.3)	9 (3.6)	10 (3.1)

Definition of abbreviations: NSCLC = non–small cell lung cancer; WHO = World Health Organization.
Values shown are numbers (%).

estimated OR of developing ILD for gefitinib treatment when stratifying by the propensity score was 3.3 (95% CI, 1.9–5.5), very similar to the primary result, suggesting that the primary regression modeling approach well captured the confounding in the data.

If some misclassification of ILD diagnosis remains despite the design features aimed to minimize it, the adjusted OR point estimate of 3.2 may apart from random variation be subject to systematic bias. A sensitivity analysis to evaluate the possible magnitude of such bias due to misclassification of ILD diagnosis suggested that the true study point estimate for the adjusted OR would be expected to lie between 2.6 and 4.8, assuming diagnostic sensitivity of more than 80% for both gefitinib- and chemotherapy-treated patients, and specificity of more than 99.0% for gefitinib and more than 99.5% for chemotherapy. Lower values for sensitivity/specificity were considered very unlikely for this serious condition in a cancer patient population, in this study setting.

Analysis of ILD Mortality

Mortality due to ILD among gefitinib- or chemotherapy-treated patients. The mortality due to ILD for the patients who developed acute ILD was 31.6% (95% CI, 21.6–43.1) among gefitinib-treated patients and 27.9% (95% CI, 15.3–43.7) among those with other treatments; the OR was 1.05 (95% CI, 0.3–3.2) for gefitinib versus chemotherapy, adjusted for relevant risk factors. Several other factors were strong predictors of a fatal outcome for patients with ILD, including age of 65 years or older, smoking history, preexisting ILD, CT scan evidence of reduced normal lung (≤50%), and/or extensive areas adherent to pleura (≥50%), with ORs ranging from 2.4 to 11.7 (*see* Table E2).

Overall mortality among gefitinib-treated patients. In the gefitinib-treated cohort in whom such data were available, an analysis of mortality from all causes by the Kaplan-Meier method showed that cumulative mortality at 12 weeks among the patients who did develop ILD was 58.7%, compared with 14.6% (95% CI, 12.8–16.3) among the large majority who did not develop ILD (Figure 3). For the entire gefitinib cohort, including the subjects who developed ILD, the observed cumulative mortality was 16.0% (95% CI, 14.3–17.8), so that the increased mortality in ILD cases impacted the total survival rate at 12 weeks in the overall gefitinib-treated cohort only to a limited extent, reducing survival from 85.4 to 84.0%.

SAEs among Gefitinib-treated Patients

SAEs were only collected for gefitinib-treated patients in the cohort, and a total of 198 patient registrations reported SAEs (10.5%), of which 38 (2.0%) reported SAEs resulting in a fatal outcome. Within this group, there were 142 patient registrations with drug-related (as reported by the physicians) SAEs (7.5%), of which 30 (1.6%) resulted in a fatal outcome. The majority of these (25 out of 30) were due to ILD-type events. There were 122 patient registrations where study treatment was discontinued due to the reported SAEs (6.5%). SAEs seen in the gefitinib-treated patients were generally consistent with the known safety profile of gefitinib and/or the patient's underlying disease and comorbidities.

DISCUSSION

This study provides important information on ILD in an advanced/recurrent NSCLC setting in Japanese patients in Japan, and it is the largest prospective study of this condition

TABLE 3. CHARACTERISTICS OF CONFIRMED CASES AND CONTROLS (AS A REPRESENTATIVE SAMPLE OF THE STUDY COHORT)

	Cases (*n* = 115)	Controls (*n* = 520)	Gefitinib Control Sample (*n* = 240)	Chemotherapy Control Sample (*n* = 280)
Severity of preexisting interstitial lung disease on CT scan (CRB evaluation)				
No ILD	84 (73.0)	473 (91.0)	231 (96.3)	242 (86.4)
Mild	15 (13.0)	28 (5.4)	8 (3.3)	20 (7.1)
Moderate	12 (10.4)	14 (2.7)	1 (0.4)	13 (4.6)
Severe	4 (3.5)	5 (1.0)	0 (0.0)	5 (1.8)
Severity of preexisting pulmonary emphysema on CT scan (CRB evaluation)				
No emphysema	56 (48.7)	326 (62.8)	176 (73.3)	150 (53.8)
Mild	35 (30.4)	92 (17.7)	36 (15.0)	56 (20.1)
Moderate	18 (15.7)	59 (11.4)	16 (6.7)	43 (15.4)
Severe	6 (5.2)	42 (8.1)	12 (5.0)	30 (10.8)
Extent of normal lung on CT scan (CRB evaluation)				
Low (10–50%)	49 (42.6)	133 (25.6)	56 (23.3)	77 (27.5)
Normal (60–100%)	66 (57.4)	387 (74.4)	184 (76.7)	203 (72.5)

Definition of abbreviations: CRB = case review board; ILD = interstitial lung disease.
Values shown are numbers (%) of total subjects with available CRB data.

to date. For the first time, the risk of acute ILD events for a large and relatively unselected chemotherapy-treated NSCLC patient cohort in Japan was determined in clinical practice. The study also quantified the greater risk of developing acute ILD associated with gefitinib treatment than with conventional chemotherapy, mainly in the first 4 weeks after treatment initiation. The study confirmed and further defined risk factors for developing ILD with gefitinib or chemotherapy. The factors included older age, poor WHO PS, smoking, short duration since diagnosis of NSCLC, reduced normal lung on CT scan, preexisting ILD, and concurrent cardiac disease. Several of these factors, or related factors, had been reported previously in bivariate or multivariate analyses from other studies (8, 18, 19). These risk factors were the same for patients treated with gefitinib or chemotherapy in the study, and no treatment-specific risk factors were identified. In particular, patients with CT evidence of preexisting ILD (chronic) were at considerably elevated risk of developing acute ILD during treatment, but there were relatively few subjects with preexisting ILD and the data did not indicate a statistically significant difference in treatment-related risk depending on the preexisting ILD status. Of clinical relevance, some of these risk factors were just as strong as, or stronger than, gefitinib treatment, for example having a poor WHO PS (≥2) rather than a good PS (OR, 4.0; 95% CI, 1.85–8.75), implying that they can be used to identify patients at particular risk of ILD in clinical practice. The relationship between ILD and pharmacokinetic characteristics of gefitinib, as well as genetic polymorphisms and proteomics determined in

TABLE 4. MEASURES OF DISEASE OCCURRENCE FOR ACUTE INTERSTITIAL LUNG DISEASE ESTIMATED FROM THE COHORT DATA (INCIDENCE RATE, CUMULATIVE INCIDENCE)

	Gefitinib Cohort	Chemotherapy Cohort
Overall observed incidence rate 0–84 d		
No. of treatment periods at Day 0	1,872	2,551
Cases of ILD/person-weeks	79/17,740	43/25,224
Incidence rate per week (95% CI)	0.00445 (0.00347–0.00544)	0.00170 (0.00120–0.00221)
Overall observed incidence rate 0–28 d		
No. of treatment periods at Day 0	1,872	2,551
Cases of ILD / person-weeks	56/7,032	21/9,902
Incidence rate per week (95% CI)	0.00796 (0.00588–0.01005)	0.00212 (0.00121–0.00303)
Overall observed incidence rate 29–56 d		
No. of treatment periods at Day 29	1,596	2,284
Cases of ILD/person-weeks	11/5,797	15/8,392
Incidence rate per week (95% CI)	0.00190 (0.00078–0.00302)	0.00179 (0.00088–0.00269)
Overall observed incidence rate 57–84 d		
No. of treatment periods at Day 57	1,328	1,890
Cases of ILD/person-weeks	12/4,911	7/6,930
Incidence rate per week (95% CI)	0.00244 (0.00106–0.00383)	0.00101 (0.00026–0.00176)
Naive cumulative incidence after 84 d (first treatment periods only)		
Cases of ILD/no. of patients	59/1,482	35/1,677
Cumulative incidence (95% CI)	3.98% (3.04–5.11%)	2.09% (1.46–2.89%)
Kaplan-Meier cumulative incidence after 84 d (first treatment periods only)		
Cases of ILD/no. of patients	59/1,482	35/1,677
Cumulative incidence (95% CI)	4.50% (3.37–5.64%)	2.40% (1.61–3.20%)

Definition of abbreviations: ILD = interstitial lung disease; CI = confidence interval.

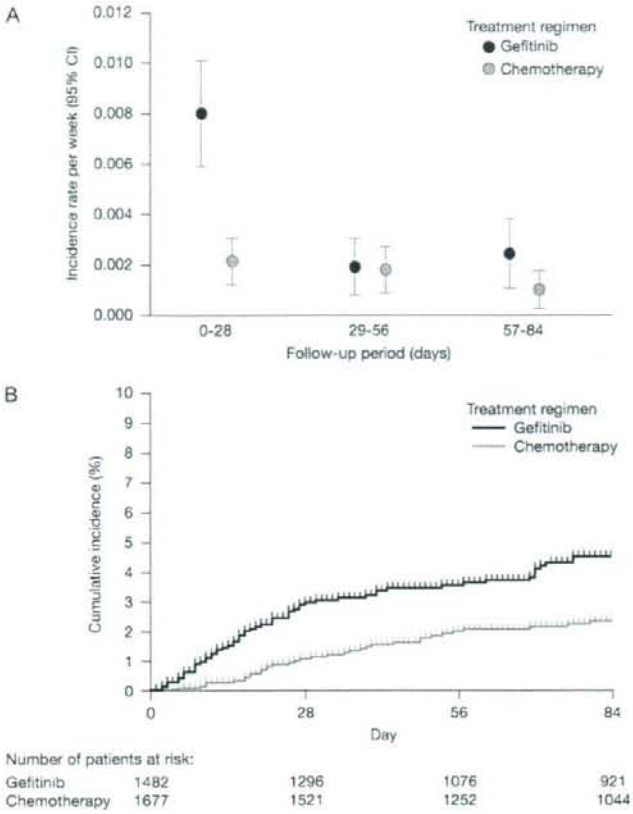


Figure 1. (*A*) Incidence rates of acute interstitial lung disease (ILD) in Japanese patients with non–small cell lung cancer for gefitinib and chemotherapy cohorts by 4-week period after treatment initiation. (*B*) Kaplan-Meier curves of risk of ILD to 12 weeks for the observed cohorts. CI = confidence interval.

study subjects, were also investigated as secondary and exploratory objectives in this study. These analyses are ongoing and results will be submitted for publication in due course.

Over the whole study follow-up, the average incidence rate for acute ILD events in patients treated with gefitinib was 3.2-fold higher relative to that seen with other chemotherapy treatments, adjusted for imbalances in other risk factors between treatments. The increased risk of ILD associated with gefitinib treatment was seen most clearly in the first 4 weeks after treatment initiation. Thus, increased physician awareness of risk factors and careful surveillance of Japanese patients during this period are indicated to manage risk. Such an approach is in line with current recommendations in Japan (20, 21). Beyond 4 weeks after treatment initiation, the risk of ILD associated with gefitinib treatment appears to fall.

ILD risk factors were found to be the same for both types of NSCLC therapy. Gefitinib is, however, a molecularly targeted agent. There is a significant body of evidence to indicate that gefitinib is a valid treatment option for some patients with NSCLC. In the IRESSA Survival Evaluation in Lung cancer (ISEL) study, a large phase III, placebo-controlled trial (n = 1,692), gefitinib was associated with some improvement in overall survival versus placebo, although this failed to reach statistical significance in the primary analysis of the overall population (22). Preplanned subgroup analyses from the study showed statistically significant differences in survival in favor of gefitinib in patients of Asian origin and those who had never smoked. Furthermore, tumor biomarker data suggest that patients with a high EGFR gene copy number, or an EGFR mutation, may be more likely to benefit (23, 24).

Therefore, the consideration of those patients more likely to benefit from the drug balanced with the better identification of these risk factors associated with ILD enables the physician to make careful judgment of the most appropriate therapy for the individual patient. Patients with several risk factors will generally be at more risk, and patients with risk factors may be at higher risk if gefitinib is used. This approach is facilitated by the fact that evidence to date suggests that subgroups less at risk of ILD tend to be those that respond well to gefitinib treatment (8).

A fatal outcome is the major concern with ILD as an SAE of drug treatment. In other large studies, fatality rates due to ILD in gefitinib-treated subjects of approximately 30% have been seen (8, 25), and a similar mortality was observed in this study in both gefitinib-treated and chemotherapy-treated ILD cases. The main predictors of a fatal outcome were older age (≥65 yr), smoking history, and preexisting ILD, as well as CT scan evidence of reduced normal lung (≤50%) or extensive areas adherent to pleura (≥50%). Because mortality is high among patients with NSCLC and the frequency of ILD in Japanese patients with NSCLC is low in comparison, ILD-related mortality impacted the overall survival at 12 weeks, for the cohort of

TABLE 5. RISK FACTORS FOR ACUTE ILD IDENTIFIED IN THE STUDY AND ESTIMATED ODDS RATIOS

Risk Factors	Odds Ratio (95% CI)
Treatment: gefitinib vs. chemotherapy	3.23 (1.94–5.40)
Age: ≥55 vs. ≤54 yr	1.92 (0.91–4.09)
WHO performance status	
1 vs. 0	1.57 (0.83–2.97)
2–3 vs. 0	4.02 (1.85–8.74)
Duration of NSCLC	
0.5 to <1 vs. <0.5 yr	0.65 (0.37–1.14)
≥1 vs. <0.5 yr	0.35 (0.20–0.62)
Concurrent cardiac disease: yes vs. no	2.44 (0.88–6.80)
Severity of preexisting pulmonary emphysema	
Mild vs. no	1.57 (0.89–2.79)
Moderate vs. no	1.04 (0.49–2.23)
Severe vs. no	0.47 (0.16–1.40)
Never-smoker and high extent of normal lung on CT (60–100%) (reference)	1.00 (reference)
Never-smoker and reduced extent of normal lung on CT (10–50%)	7.22 (2.52–20.64)
Smoker and high extent of normal lung on CT (60-100%)	4.43 (1.87–10.47)
Smoker and reduced extent of normal lung on CT (10–50%)	5.42 (2.08–14.12)
No preexisting ILD and high extent of normal lung on CT (60–100%) (reference)	1.00 (reference)
No preexisting ILD and reduced extent of normal lung on CT (10–50%)	7.22 (2.52–20.64)
Mild preexisting ILD and high extent of normal lung on CT (60–100%)	4.80 (1.83–12.63)
Mild preexisting ILD and reduced extent of normal lung on CT (10–50%)	6.08 (1.09–33.98)
Moderate–severe preexisting ILD and high extent of normal lung on CT (60–100%)	5.55 (1.40–21.99)
Moderate–severe preexisting ILD and reduced extent of normal lung on CT (10–50%)	25.27 (5.74–111.28)

Definition of abbreviations: CI = confidence interval; CT = computed tomography; ILD = interstitial lung disease; NSCLC = non–small cell lung cancer; WHO = World Health Organization.

gefitinib-treated patients, only to a limited extent (85.4 to 84%). Accordingly, there needs to be an appropriate individualized risk–benefit evaluation for patients also considering other treatments, many of which have their own problems with treatment-related mortality due to SAEs other than ILD.

Some methodologic issues may have influenced the study results and deserve comment. This kind of observational pharmacoepidemiologic study is generally considered sensitive to confounding by indication. Most often, it is assumed that more "sick" or "susceptible" patients will receive a new treatment,

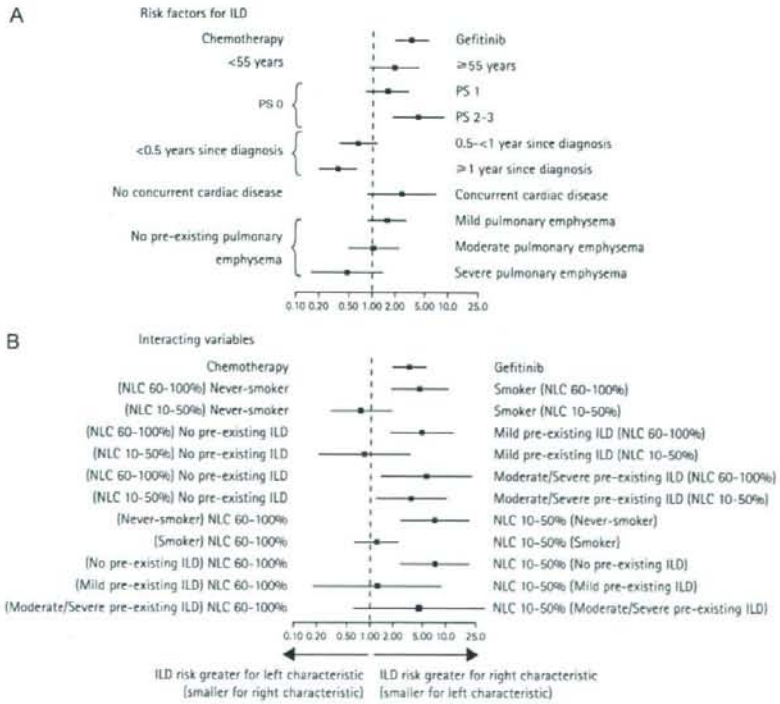
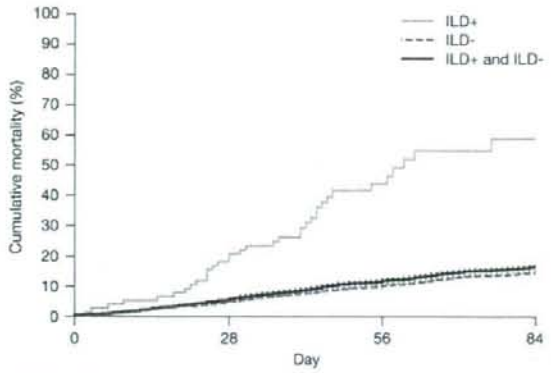


Figure 2. Adjusted odds ratios for risk factors for acute interstitial lung disease (ILD) in Japanese patients with non–small cell lung cancer from final logistic model. NLC = normal lung coverage (extent of normal lung on computed tomography scan); PS = World Health Organization performance status.



Number of patients at risk:

ILD+	78	64	22	11
ILD-	1771	1694	1416	1054
ILD+ and ILD-	1849	1758	1438	1065

Figure 3. Kaplan-Meier curves showing risk of death to 12 weeks in the gefitinib cohort overall and subdivided into those that developed interstitial lung disease (ILD+) and those who did not (ILD−).

leading to possibly more adverse effects in this group, even in the absence of a true relationship to treatment. Attempts to adjust for confounding using collected data would then push the adjusted estimate of effect closer to the null, but if sufficiently precise information on strong confounders cannot be collected, it may be impossible to remove all of the confounding. In conducting this study, the suspected adverse effect of ILD was recognized, and in the clinical setting, recommendations were in place to proceed with caution when treating some patients with suspected elevated baseline risk of ILD. This kind of selection would tend to produce the type of data pattern that was in fact observed in this study, a pattern of negative confounding that produces a more elevated OR when adjustment for confounders is performed. Thus, the results are well in line with what might be expected.

Misdiagnosis of ILD (outcome misclassification) is another concern, but it is expected that the stringent design features have minimized this problem in the present study (*see* online supplement for details). The diagnostic CRB review is a key feature, but it was still CT based, and biopsies—generally considered the gold standard for ILD diagnosis—were in most cases not taken. Overall, a sensitivity analysis suggested that, under reasonable assumptions about possible misclassification of ILD, the main result would remain similar and the conclusions from the study would not be greatly changed.

Random error is another consideration. However, although random error may be responsible for some bias in the point estimate, the confidence interval is reasonably narrow. The results are also consistent with other recent data. For example, as of January 2006, the estimated reporting rate of ILD-type events in Japan from the AstraZeneca Global Drug Safety Database of patients receiving gefitinib treatment was approximately 3.1% (26); from a retrospective study by the West Japan Thoracic Oncology Group (WJTOG), which studied 1,719 patients receiving gefitinib of whom 69 developed ILD, the frequency was 3.5% (95% CI, 2.8–4.5%) (8); from a postmarketing surveillance (PMS) study conducted by AstraZeneca KK Japan, which included 3,322 gefitinib-treated patients, it was 5.8% (25); whereas from the present study, the cumulative incidence at 12 weeks was 4.0% (95% CI, 3.0–5.1%).

These estimates are quite similar, even recognizing that the populations and selection of patients differ between these samples, and duration of follow-up, although similar, varies. In the present study, for the first time, an estimate of cumulative incidence of ILD after 12 weeks of treatment was obtained also from a chemotherapy-treated patient group; this frequency was 2.1% (95% CI, 1.5–2.9%), providing an estimate of this problem unrelated to gefitinib in patients with NSCLC in Japan.

The prognosis for gefitinib-treated patients who were diagnosed with ILD was also quite consistent with other studies. In the PMS study, ILD-related death among patients diagnosed with ILD was 38.6% (25); in the WJTOG study it was 44.3% (8); in the AstraZeneca Global Drug Safety Database as of January 2006, the proportion of ILD-type events with a fatal outcome in patients receiving treatment with gefitinib in Japan was 37.3% (AstraZeneca, data on file); and in the present study it was 31.6%. This proportion was quite similar to the chemotherapy-treated group, 27.9% (adjusted OR, 1.05; 95% CI, 0.4–3.2).

The factors associated with risk of acute ILD observed in this Japanese NSCLC population are largely different or even complementary to factors that predict better response to gefitinib. This would seem to support a hypothesis that the mechanism by which ILD occurs is distinct from the successful cancer response mechanism, offering a potential path toward selecting patients with optimal risk–benefit balance for gefitinib treatment.

Interestingly, the issue of ILD in patients with NSCLC, after gefitinib or other treatments, appears to be a problem largely limited to Japan. From the AstraZeneca Global Drug Safety Database, the reporting rate of ILD-type events in patients receiving treatment with gefitinib was only 0.23% in the rest of the world excluding Japan, based on more than 215,000 patients worldwide estimated to have been exposed to gefitinib (26). Even for neighboring countries, the pattern differs from Japan: the rate for East Asian countries, including Korea and Taiwan but excluding Japan, was 0.17% (26). The proportion of ILD-type events with a fatal outcome was similar, however: 37% in Japan and 31% in the rest of the world. The reasons for this difference in incidence of ILD between Japan and other countries remain unclear, but may relate to both constitutional and environmental factors specific to Japan or Japanese patients. For other drug treatments, too, a higher incidence of ILD has been noted in Japan than elsewhere (12, 13).

Within the study, some exploratory analyses are still ongoing related to genetic and proteomic predictors for ILD in patients with NSCLC, to search for biomarkers for early recognition of ILD and hopefully individualized risk assessment. This may

help to shed light on why ILD appears to be a particular issue for Japanese patients and the possible underlying mechanisms.

The EGFR is expressed on a number of constituent cells of the lungs including epithelium, smooth muscle cells, fibroblasts, and endothelium (27). There have been a number of animal studies using bleomycin- and vanadium pentoxide–induced lung injury with EGFR–tyrosine kinase inhibitors to determine the role of EGFR in lung fibrosis. Gefitinib and AG1478 have been used in such studies of mice and, when administered in a range of therapeutic doses, show clear attenuation of both bleomycin- (28) and vanadium pentoxide–induced (29) lung fibrosis, although one study (30) has shown augmentation of bleomycin-induced fibrosis (when using a subtoxic dose of gefitinib). The similarity of study design and choice of animal strain in the bleomycin studies make it difficult to explain the discrepant results other than by the excessive dosing. This leaves uncertainty as to the underlying mechanism of lung fibrosis in patients with NSCLC receiving gefitinib.

In summary, the study appears to be of adequate validity to avoid serious systematic biases, random error does not seem to be the most likely explanation for the results, and the observed increased risk of ILD with gefitinib treatment relative to chemotherapy treatment in Japanese patients is consistent with previous studies. Although preexisting ILD was confirmed as an important determinant of developing acute ILD symptoms after treatment with gefitinib or chemotherapy, the results also suggested that risk of ILD may be generally affected by a variety of other factors that decrease the amount of normally functioning lung tissue or affect the capability of tissue repair and recovery. The study thus identified several risk factors apart from treatment, which included preexisting ILD, which were not treatment specific, and which were partly similar to risk factors for idiopathic or rheumatic pulmonary lung fibrosis. These findings taken together suggest that there may be a common etiology that gives some patients a greater susceptibility both to idiopathic or rheumatic pulmonary fibrosis and to acute drug-induced lung injury after various treatments.

Conflict of Interest Statement: S.K. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. H.K. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. Y.N. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. M.F. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. K.N. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. Y.I. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. M.T. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. S.Y. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. K.N. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. M.S. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. The Japan Thoracic Radiology Group does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. H.J. has been an AstraZeneca employee since 2001. Y.I. has been an AstraZeneca employee since 2000. A.A. is a full-time employee of AstraZeneca. C.W. has been a full-time employee at AstraZeneca since 2001 until present and owns shares in the company. T.H. is a full-time R&D scientist at AstraZeneca, UK, and received stock options. F.N. is a full-time employee of AstraZeneca and owns shares in the company.

Acknowledgment: The authors thank the study monitors, nurses, data managers, other support staff, and the patients participating in the study; the external epidemiology advisory board (Kenneth J. Rothman, Jonathan M. Samet, Toshiro Takezaki, Kotaro Ozasa, Masahiko Ando) for their advice and scientific review of study design, conduct, and analysis; Professor Nestor Müller for his expert input into radiologic aspects of ILD diagnosis; all case review board members (including Japan Thoracic Radiology Group members [with *]) individually (Moritaka Suga, Takeshi Johkoh*, Masashi Takahashi*, Yoshiharu Ohno*, Sonoko Nagai, Yoshio Taguchi, Yoshikazu Inoue, Takashi Yana, Masahiko Kusumoto*, Hiroaki Arakawa*, Akinobu Yoshimura, Makoto Nishio, Yuichiro Ohe, Kunihiko Yoshimura, Hiroki Takahashi, Yukihiko Sugiyama, Masahito Ebina, and Fumikazu Sakai*) for their valuable work in blindly reviewing ILD diagnoses, as well as prestudy CT scans for preexisting comorbidities; and all hospitals and clinical investigators who contributed to the data collection in the study (*see below*). The authors thank Sarah Charlesworth, from Complete Medical Communications, who provided editing assistance funded by AstraZeneca.

Hospitals and principal investigators contributing to the study: National Hospital Organization Hokkaido Cancer Centre (Hiroshi Isobe), Hokkaido University Hospital (Koichi Yamazaki), National Hospital Organization Dohoku National Hospital (Yuka Fujita), Tohoku University Hospital (Akira Inoue), Sendai Kousei Hospital (Shunichi Sugawara), National Cancer Centre Hospital East (Yutaka Nishiwaki), Nippon Medical School Chiba Hokusoh Hospital (Yasushi Ono), Tokyo Medical University Hospital (Masahiro Tsuboi), Nippon Medical School Hospital (Tetsuya Okano), Toho University Omori Medical Centre (Nobuyuki Hamanaka), Toranomon Hospital (Kunihiko Yoshimura), National Hospital Organization Tokyo Hospital (Atsuhisa Tamura), Juntendo University Hospital (Kazuhisa Takahashi), Kyorin University Hospital (Tomoyuki Goya), Tokai University Hospital (Kenji Eguchi), Kitasato University School of Medicine (Noriyuki Masuda), Kanagawa Cardiovascular and Respiratory Centre (Takashi Ogura), Niigata Cancer Centre Hospital (Akira Yokoyama), National Nishi-Niigata Central Hospital (Hiromi Miyao), Toyama University Hospital (Muneharu Maruyama), Kanazawa University Hospital (Kazuo Kasahara), Aichi Hospital, Aichi Cancer Centre (Hiroshi Saito), National Hospital Organization Nagoya Medical Centre (Hideo Saka), Fujita Health University Hospital (Hiroki Sakakibara), Nagoya Ekisaikai Hospital (Masashi Yamamoto), Shiga University of Medical Science Hospital (Noriaki Tezuka), Kyoto Katsura Hospital (Takeshi Hanawa), National Hospital Organization Kyoto Medical Centre (Yoshiyuki Sasaki), Rinku General Medical Centre Municipal Izumisano Hospital (Hisao Uejima), Kinki University, School of Medicine (Kazuhiko Nakagawa), National Hospital Organization Kinki-chuo Chest Medical Centre (Masaaki Kawahara), Osaka City General Hospital (Koji Takeda), Osaka City General Hospital (Hirohito Tada), Osaka City University Hospital (Shinzoh Kudoh), Osaka Prefectural Medical Centre for Respiratory and Allergic Diseases (Kaoru Matsui), Osaka Police Hospital (Kiyoshi Komuta), Toneyama National Hospital (Soichiro Yokota), Kobe City General Hospital (Keisuke Tomii), Hyogo Medical Centre for Adults (Shunichi Negoro), Kobe University Hospital (Yoshihiro Nishimura), Institute of Biomedical Research and Innovation (Nobuyuki Katakami), Tenri Hospital (Yoshio Taguchi), Okayama University Medical and Dental School Hospital (Katsuyuki Kiura), Hiroshima City Hospital (Hidetaka Sumiyoshi), Hiroshima City Hospital (Noritomo Senoo), National Hospital Organization Shikoku Cancer Centre (Tetsu Shinkai), National Hospital Organization Kyushu Cancer Centre (Yukito Ichinose), Fukuoka National Hospital (Akira Motohiro), University of Occupational and Environmental Health (Masamitsu Kido), University of Occupational and Environmental Health (Kenji Sugio), National Hospital Organization Nagasaki Medical Centre (Akitoshi Kinoshita), Kumamoto University Hospital (Mitsuhiro Matsumoto), Kumamoto-Chuo Hospital (Sunao Ushijima), Okinawa National Hospital (Mutsuo Kuba).

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Association of epidermal growth factor receptor (*EGFR*) gene mutations with *EGFR* amplification in advanced non-small cell lung cancer

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(Received May 28, 2008/Revised August 5, 2008/Accepted August 6, 2008/Online publication October 16, 2008)

Somatic mutations in the epidermal growth factor receptor (*EGFR*) gene are associated with the response to *EGFR* tyrosine kinase inhibitors in patients with non-small cell lung cancer (NSCLC). Increased *EGFR* copy number has also been associated with sensitivity to these drugs. However, given that it is often difficult to obtain sufficient amounts of tumor tissue for genetic analysis from patients with advanced NSCLC, the relationship between these two types of *EGFR* alterations has remained unclear. We have now evaluated *EGFR* mutation status both by direct sequencing and with a high-sensitivity assay, the Scorpion-amplification-refractory mutation system, and have determined *EGFR* copy number by fluorescence *in situ* hybridization (FISH) analysis in paired tumor specimens obtained from 100 consecutive patients with advanced NSCLC treated with chemotherapy. *EGFR* mutations or FISH positivity (*EGFR* amplification or high polysomy) were apparent in 18% (18/100) and 32% (32/100) of patients, respectively. The Scorpion-amplification-refractory mutation system was more sensitive than direct sequencing for the detection of *EGFR* mutations. Furthermore, *EGFR* mutations were associated with *EGFR* amplification ($P = 0.009$) but not with FISH positivity ($P = 0.266$). Our results therefore suggest the existence of a significant association between *EGFR* mutation and *EGFR* amplification in patients with advanced NSCLC. (*Cancer Sci* 2008; 99: 2455–2460)

The epidermal growth factor receptor (EGFR) is a receptor tyrosine kinase of the ErbB family and has been implicated in the proliferation and survival of cancer cells. Aberrant expression of *EGFR* has been detected in many human epithelial malignancies, including non-small cell lung cancer (NSCLC).[1,2] This receptor has therefore been identified as a promising target for anticancer therapy, and several agents have been synthesized that inhibit its tyrosine kinase activity. *EGFR* tyrosine kinase inhibitors (TKI) have been evaluated most extensively in individuals with NSCLC, and they have had a substantial impact on the treatment of this disease by offering additional therapeutic options for patients with advanced NSCLC.[3–6]

Somatic mutations in the tyrosine kinase domain of *EGFR* have been detected in a subset of NSCLC patients who respond to EGFR TKI[7–9] and have been shown to be closely associated with sensitivity to these drugs.[10–14] Indeed, we and others have prospectively demonstrated a high response rate to EGFR TKI therapy in NSCLC patients with *EGFR* mutations.[15–21] An increased copy number of the *EGFR* gene, as revealed by fluorescence *in situ* hybridization (FISH), has also emerged as an effective molecular marker of EGFR TKI sensitivity in NSCLC.[22–24] We previously showed that *EGFR* mutation and *EGFR* amplification are associated in human NSCLC cell lines and that endogenous EGFR expressed in such cell lines positive for both of these *EGFR* alterations are activated constitutively.[25] However, the relationship between *EGFR* mutation and FISH positivity for *EGFR*, which reflects gene amplification or high polysomy, has remained unclear.[22–24,26,27] Indeed, only a few studies have evaluated the relationship between mutation and gene copy number for *EGFR* because of the difficulty in obtaining tumor samples suitable for genetic analysis from individuals with advanced NSCLC. We previously showed that the Scorpion-amplification-refractory mutation system (ARMS) is a sensitive technique for the detection of *EGFR* mutations in tumor specimens such as pleural effusion fluid or tissue obtained by transbronchial needle aspiration.[28–30] In the present study, we evaluated *EGFR* mutation status in small tumor specimens from patients with advanced NSCLC both by direct sequencing and by Scorpion-ARMS and compared the sensitivity of these methods for the detection of *EGFR* mutations. Furthermore, we determined *EGFR* copy number by FISH analysis in paired tumor specimens and examined its relationship to *EGFR* mutation.

Materials and Methods

Patients. The present retrospective study recruited consecutive patients with advanced NSCLC who received chemotherapy at Kinki University Hospital between January 2003 and December 2005. Patients eligible for the study had histologically confirmed stage III or IV NSCLC that was not curable by surgical resection or radiotherapy, irrespective of the presence of measurable lesions or good performance status (PS). Patients with recurrence after surgical resection were excluded. Complete clinical information and tissue blocks suitable for genetic analysis were available for 100 patients. We examined the relationship between *EGFR* mutation and *EGFR* copy number as well as the influence of these *EGFR* alterations on clinical outcome. Tumor response was assessed by computed tomography and evaluated according to the Response Evaluation Criteria in Solid Tumors.[31] Survival was calculated from the date of initiation of chemotherapy either to the date of death from any cause or to the date of last contact. Some patients had been receiving EGFR TKI treatment before the demonstration in 2004 that mutations in *EGFR* confer increased sensitivity to these drugs. Moreover, many patients had already died before the initiation of our genetic analysis, preventing us from obtaining informed consent. The institutional review board

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doi: 10.1111/j.1349-7006.2008.00962.x

therefore approved our study protocol with the conditions that samples would be processed anonymously and analyzed only for somatic mutations (not for germline mutations) and that the study would be disclosed publicly, according to the Ethical Guidelines for Human Genome Research published by the Ministry of Education, Culture, Sports, Science, and Technology, the Ministry of Health, Labor, and Welfare, and the Ministry of Economy, Trade, and Industry of Japan. The present study also conforms to the provisions of the Declaration of Helsinki.

Identification of *EGFR* mutations. The tumor specimens were fixed with formalin and embedded in paraffin. DNA was extracted with the use of a QIAamp Micro kit (Qiagen K.K., Tokyo, Japan) from tumor tissue derived either by macrodissection or by laser-capture microdissection carried out to enrich tumor cells. Polymerase chain reaction-based direct sequencing of exons 18–21 and ARMS with designed 'Scorpion' primers were applied for the allele-specific detection of *EGFR* mutations. Only the following previously described mutations[7,8] were classified as mutations in the present study: G719X in exon 18, deletion of E746 to A750 or of neighboring residues in exon 19, as well as L858R and L861Q in exon 21. Patients were regarded as *EGFR* mutation positive if a mutation in *EGFR* was detected either by direct sequencing or by ARMS. All mutations were confirmed by analysis of at least two independent amplification products.

Determination of *EGFR* copy number. *EGFR* copy number was determined by FISH analysis with the use of dual-color DNA probes (LSI *EGFR* SpectrumOrange/CEP 7 SpectrumGreen; Vysis, Downers Grove, IL, USA). The tumor specimens were classified into six categories on the basis of the FISH results, as described previously.[22] Those with high polysomy (≥4 copies of *EGFR* in ≥40% of cells) or gene amplification (presence of a tight *EGFR* gene cluster and a ratio of *EGFR* to chromosome 7 of ≥2 or ≥15 copies of *EGFR* per cell in ≥10% of cells analyzed) were considered FISH positive, with those in the remaining categories being considered FISH negative.

Statistical analysis. The relationships among *EGFR* status, clinical characteristics, and tumor response to EGFR TKI were analyzed with Fisher's exact test as appropriate. Survival curves were constructed by the Kaplan–Meier method, and the differences in survival between patient subgroups were compared by the log-rank test. The impact of various factors on survival was evaluated by univariate and multivariate analysis according to the Cox regression model. A P-value <0.05 was considered statistically significant. All statistical analysis was carried out with StatView software (SAS Institute, Cary, NC, USA).

Results

Patient characteristics. Between January 2003 and December 2005, a total of 125 consecutive patients diagnosed histologically with advanced NSCLC underwent chemotherapy at Kinki University Hospital. Tissue specimens from 100 patients were assessable for both *EGFR* mutation and *EGFR* copy number. Of these specimens, 72 were obtained by bronchoscopic biopsy, 15 by percutaneous needle biopsy (12 from lung, two from bone, and one from lymph node), six by thoracoscopic biopsy, and seven by surgery for diagnosis or palliative therapy. The clinical characteristics of these 100 patients are shown in Table 1. Most of the patients were male (64%) and had a history of smoking (67%), and adenocarcinoma was the most prevalent tumor histology (61%). Most patients (83%) also had a good Eastern Cooperative Oncology Group PS (0 or 1), and 63% received second-line or subsequent rounds of chemotherapy. Fifty-three patients (53%) were treated with EGFR TKI. Seventy patients (70%) had died by the time of genetic analysis, with the median follow-up time for the 30 survivors being 14.6 months.

***EGFR* alterations in non-small cell lung cancer.** Patients were analyzed for *EGFR* mutations by direct sequencing of exons 18 through 21 and by Scorpion-ARMS (Table 2). *EGFR* mutations, consisting of in-frame deletions in exon 19 ($n = 5$) and point mutations in exon 21 ($n = 13$), were detected in 18 patients (18%). Eight *EGFR* mutations were detected by direct sequencing and 16 mutations were detected by Scorpion-ARMS. Ten of the 16 mutations detected by Scorpion-ARMS were not identified by direct sequencing. However, two of the deletions in exon 19 (E746_S752 and E746_T751) that were detected by direct sequencing were not identified by Scorpion-ARMS, given that the Scorpion primers were designed only for detection of the E746_A750 deletion in exon 19. *EGFR* mutations were significantly more frequent in tumors of women than in those of men (33 vs 9%), in adenocarcinomas than in tumors with other histologies (28 vs 3%), and in never-smokers than in smokers (42 vs 6%) (Fig. 1a). One of the 18 *EGFR* mutations was detected in a squamous cell carcinoma. Determination of *EGFR* copy number by FISH analysis revealed gene amplification in six patients and high polysomy in 26 patients, with 32 patients thus being classified as FISH positive (Table 3). In contrast to *EGFR* mutation, FISH

Table 1. Characteristics of patients with advanced non-small cell lung cancer ($n = 100$)

Characteristic	Subset	No. patients
Sex	Male	64
	Female	36
Smoking history	Never-smoker	33
	Smoker	67
Tumor histology	Adenocarcinoma	61
	Other	39
Eastern Cooperative Oncology Group performance status	0	24
	1	59
	≥2	17
No. chemotherapies	1	37
	≥2	63

Table 2. Detection of epidermal growth factor receptor (*EGFR*) mutations by direct sequencing or amplification-refractory mutation system (ARMS) ($n = 100$)

Site	Mutation	Direct sequencing	ARMS	Direct sequencing or ARMS
Exon 19	15-bp deletion	1	3	3
	16-bp deletion	1	0	1
	19-bp deletion	1	0	1
Exon 21	L858R	5	13	13
Total		8 (8%)	16 (16%)	18 (18%)

Table 3. Determination of epidermal growth factor receptor gene copy number by fluorescence *in situ* hybridization (FISH) analysis ($n = 100$)

FISH status	Finding	No. patients
Positive	Gene amplification	6
	High polysomy	26
	Total	32
Negative	Low polysomy	35
	High trisomy	2
	Low trisomy	26
	Disomy	5
	Total	68

doi: 10.1111/j.1349-7006.2008.00962.x

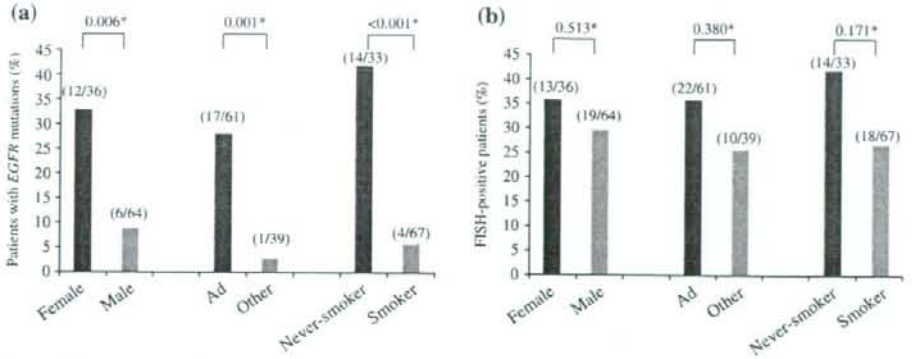

Fig. 1. Sex, tumor histology, and smoking status of patients with advanced non-small cell lung cancer and with either (a) epidermal growth factor receptor (*EGFR*) mutations or (b) a high *EGFR* copy number. Ad, adenocarcinoma. *P-values were determined by Fisher's exact test.

Table 4. Relationship between epidermal growth factor receptor (*EGFR*) mutation and either fluorescence *in situ* hybridization (FISH) status of *EGFR* amplification

Mutation status	FISH status		Gene amplification	
	Positive	Negative	Positive	Negative
Positive ($n = 18$)	8	10	4	14
Negative ($n = 82$)	24	58	2	80
P-value*		0.266		0.009

*Determined by Fisher's exact test.

positivity was not associated with sex, tumor histology, or smoking status (Fig. 1b). Although no relationship was apparent between *EGFR* mutation and FISH positivity (gene amplification or high polysomy), *EGFR* mutation and *EGFR* amplification were significantly associated (Table 4). The clinicopathological and genetic features of patients with *EGFR* mutations are shown in Table 5.

Overall survival. For the total patient population, the median overall survival was 12.3 months, with a 1-year survival rate of 51.7%. Univariate analysis revealed that overall survival was significantly longer in women, never-smokers, patients with a favorable PS, and those with *EGFR* mutations (Table 6; Fig. 2a). In contrast, no difference in overall survival was apparent between FISH-positive and FISH-negative patients (Table 6; Fig. 2b). We also carried out multivariate analysis to identify factors that contribute to overall survival, with covariates including clinicopathological and genetic factors (sex, smoking history, tumor histology, PS, *EGFR* mutation status, FISH status). Female sex and favorable PS were found to be independent prognostic factors (Table 6).

Responsiveness to epidermal growth factor receptor tyrosine kinase inhibitor treatment. Of the 53 patients treated with EGFR TKI, 40 individuals were assessable for objective response. Whereas the rate of response to EGFR TKI treatment for patients with *EGFR* mutations was significantly higher than that for those without such mutations (71.4 vs 11.5%, $P < 0.001$), there was no significant association between FISH status and responsiveness

Table 5. Clinicopathological and genetic features of patients with epidermal growth factor receptor (*EGFR*) mutations

No.	Age (years)	Sex	Smoking status	Histology	Response to EGFR TKI	Type of *EGFR* mutation		*EGFR* copy number
						Sequencing	ARMS	
1	72	F	Never	Ad	PR		L858R	Low trisomy
2	58	F	Never	Ad	PR	L858R	L858R	Gene amplification
3	81	F	Never	Ad	SD	L858R	L858R	High polysomy
4	72	F	Never	Ad	NE		L858R	Gene amplification
5	48	M	Smoker	Ad	SD		L858R	Low trisomy
6	67	F	Never	Ad	SD		L858R	Low trisomy
7	59	F	Never	Ad	PR		L858R	High polysomy
8	78	M	Smoker	Ad			L858R	High trisomy
9	71	F	Never	Ad	PR		L858R	Low polysomy
10	82	F	Never	Ad	PR	L858R	L858R	Low trisomy
11	67	F	Never	Ad		L858R	L858R	High polysomy
12	87	F	Never	Sq	PR	L858R	L858R	Low polysomy
13	78	M	Never	Ad			L858R	Gene amplification
14	56	F	Never	Ad	PR		(E746_A750)del	Low polysomy
15	63	M	Never	Ad	PD	(E746_A750)del	(E746_A750)del	Gene amplification
16	63	M	Smoker	Ad	PR		(E746_A750)del	Low polysomy
17	61	M	Smoker	Ad	PR	(E746_S752)del insV		Low trisomy
18	73	F	Never	Ad	PR	(E746_T751)del insS		High polysomy

Ad, adenocarcinoma; ARMS, amplification-refractory mutation system; NE, not evaluated; PD, progressive disease; PR, partial response; SD, stable disease; Sq, squamous cell carcinoma; TKI, tyrosine kinase inhibitor.

Table 6. Univariate and multivariate analyses of prognostic factors for overall survival

Factor	Univariate analysis			Multivariate analysis		
	HR	95% CI	*P*-value	HR	95% CI	*P*-value
Sex (female/male)	0.54	0.32–0.91	**0.021**	0.55	0.32–0.93	**0.025**
Smoking history (never-smoker/smoker)	0.50	0.30–0.85	**0.011**			
Histology (adenocarcinoma/other)	0.64	0.39–1.05	0.077	0.68	0.40–1.14	0.141
ECOG PS (0/≥1)	0.44	0.24–0.79	**0.006**	0.48	0.29–0.86	**0.019**
EGFR mutation status (positive/negative)	0.52	0.28–0.97	**0.039**			
FISH status (positive/negative)	1.36	0.82–2.23	0.231	1.49	0.88–2.50	0.130

CI, confidence interval; ECOG, Eastern Cooperative Oncology Group; EGFR, epidermal growth factor receptor; FISH, fluorescence *in situ* hybridization; HR, hazard ratio; PS, performance status. Multivariate analysis was carried out using the stepwise method (include, <0.05; exclude, >0.2). Significant *P*-values are shown in bold.

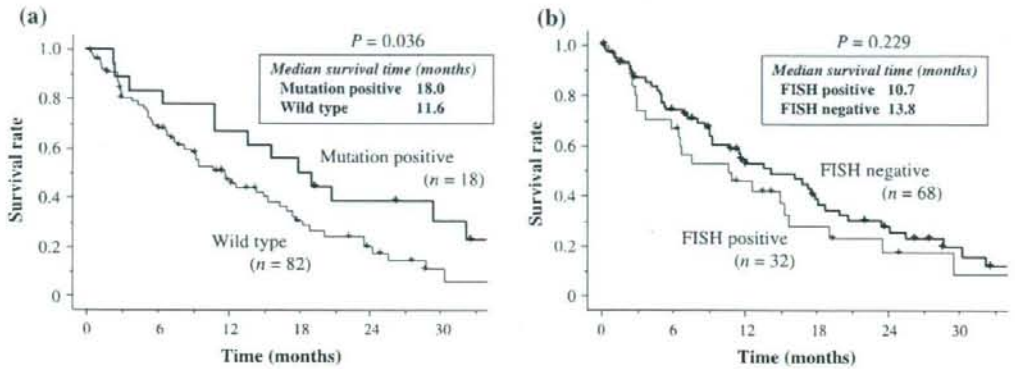

Fig. 2. Kaplan–Meier plots of overall survival in patients with advanced non-small cell lung cancer and either (a) with or without epidermal growth factor receptor (*EGFR*) mutations or (b) with or without a high *EGFR* copy number. FISH, fluorescence *in situ* hybridization.

to EGFR TKI (44.4 vs 29.0% for FISH-positive vs FISH-negative patients, respectively, $P = 0.437$).

Discussion

We have analyzed both *EGFR* mutation and *EGFR* copy number in paired tumor specimens as well as the relationship between these two types of *EGFR* alterations in advanced NSCLC. We used two methods to detect *EGFR* mutations, direct sequencing and Scorpion-ARMS, which identified eight and 16 mutations, respectively. Direct sequencing failed to detect 10 of the 16 mutations identified by Scorpion-ARMS. Of the 10 patients with *EGFR* mutations detected by Scorpion-ARMS alone, seven were assessable for an objective response to EGFR TKI, with five exhibiting a partial response and two having stable disease. Consistent with previous observations,[28–30] our data thus indicate that Scorpion-ARMS is more sensitive than direct sequencing for detection of the two major types of *EGFR* mutation that reflect responsiveness to EGFR TKI. It should be noted, however, that most polymerase chain reaction-based systems for mutation analysis, including Scorpion-ARMS, are able to detect only known *EGFR* mutations targeted by the designed primers. Indeed, two minor variants of deletion mutation in exon 19 were not identified by Scorpion-ARMS in the present study. Given the exclusion of recurrence after surgical resection in our study, most tumor specimens analyzed were obtained either by transbronchial lung biopsy or by percutaneous needle lung biopsy. The amount of tumor tissue obtained by these procedures is limited, but our results suggest that it is sufficient both for histopathological analysis and for the detection of *EGFR* mutations by Scorpion-ARMS in patients with advanced NSCLC.

Scorpion-ARMS identified three E746_A750 deletion mutations in exon 19 and 13 L858R point mutations in exon 21 in the present study. The frequency of the E746_A750 mutation detected by Scorpion-ARMS thus appeared low compared with that of the L858R mutation. Previous studies have shown that the incidence of the E746_A750 deletion is approximately the same as that of the L858R mutation.[10,12] The sensitivity of Scorpion-ARMS for detection of the E746_A750 deletion is equivalent to that for detection of the L858R point mutation. The low frequency of the E746_A750 deletion mutation in the present study is thus likely due to the small number of samples.

Previous studies have revealed a higher prevalence of *EGFR* mutations in East Asians than in Caucasians.[4,10–12,20,22,24,26,27,32–36] The prevalence of *EGFR* mutations in our Japanese cohort was low (18%) compared with values determined previously for East Asian populations. Given that most previous studies examined only individuals treated with EGFR TKI, patient selection based on clinical predictors might have led to an increase in the proportion of subjects with adenocarcinoma histology, a factor known to be associated with *EGFR* mutations. In contrast, our study was carried out with consecutive cases irrespective of EGFR TKI treatment. The relatively low proportion of patients with adenocarcinoma histology (61%) in our cohort is therefore consistent with the low prevalence of *EGFR* mutations. However, the FISH positivity of 32% in our study is similar to that in previous studies that adopted the same criteria, with values ranging from 31 to 48%.[22–24,26,27] Consistent with previous

doi: 10.1111/j.1349-7006.2008.00962.x

results,[1,7–9,12] *EGFR* mutations were significantly more frequent among women, never-smokers, and patients with adenocarcinoma in the present study. In contrast, neither *EGFR* amplification (analysis not shown) nor FISH positivity was associated with any such clinicopathological factor in our study, although the relationship between *EGFR* amplification and never-smoking status approached statistical significance ($P = 0.090$).

The relationship between *EGFR* mutation and FISH positivity (gene amplification or high polysomy) in NSCLC patients has remained unclear.[22–24,26,27] In the present study, we have demonstrated a significant relationship between *EGFR* mutation and *EGFR* amplification, but not between *EGFR* mutation and FISH positivity, in tumor specimens from patients with advanced NSCLC. *EGFR* mutant alleles were previously found to be amplified selectively, resulting in a high *EGFR* copy number, as detected by quantitative real-time polymerase chain reaction analysis.[12] *EGFR* amplification has also been shown to be acquired during invasive growth of lung adenocarcinoma with *EGFR* mutations.[37] Furthermore, recent studies have found that an increase in *EGFR* copy number is a relatively late event in NSCLC pathogenesis[38] and that *EGFR* mutation precedes *EGFR* amplification but not necessarily high polysomy.[37,39] These observations thus support the existence of a close association between *EGFR* mutation and *EGFR* amplification. We previously showed that *EGFR* mutation was significantly associated with *EGFR* amplification in human NSCLC cell lines and that endogenous EGFR expressed in such cell lines that manifested both of these *EGFR* alterations were activated constitutively as a result of ligand-independent dimerization.[25] However, the biological consequences of high polysomy for *EGFR* have not been elucidated. We did not find any cut-off value of high polysomy that was associated with *EGFR* mutation. We therefore propose that *EGFR* amplification, but not high polysomy, plays a key role in the pathogenesis of NSCLC and correlates with *EGFR* mutation.

We sought to determine whether *EGFR* mutation or *EGFR* copy number might affect overall survival of NSCLC patients. Previous studies of EGFR TKI have suggested that *EGFR* mutation is a favorable prognostic indicator for patients with NSCLC.[35,36] We also found that the survival time of patients with *EGFR* mutations was longer than that of those without them (18.0 vs 11.6 months, $P = 0.036$) in the univariate analysis. However, interpretation of this result requires that the effect of EGFR TKI on survival be taken into account, given that 83% (15/18) of patients with *EGFR* mutations were treated with EGFR TKI compared with only 46% (38/82) of those without such mutations. Indeed, analysis of survival after initiation of EGFR TKI treatment as a second-line or subsequent therapy revealed a survival time of 15.6 months for mutation-positive patients vs 6.0 months for mutation-negative patients in our study. It was therefore not possible to determine the prognostic significance of *EGFR* mutation for NSCLC patients. To clarify whether *EGFR* mutation is a predictor of sensitivity to EGFR TKI or a prognostic indicator for NSCLC patients, we are currently carrying out a phase III randomized study comparing platinum-based chemotherapy with gefitinib in chemotherapy-naive NSCLC patients with *EGFR* mutations. Patients with FISH-positive tumors tended to have a shorter survival time than did those with FISH-negative tumors (10.7 vs 13.8 months), although this difference was not statistically significant. This result is consistent with previous observations indicative of an association between high *EGFR* copy number and poor prognosis for certain malignancies, including NSCLC.[1,40]

In conclusion, we have analyzed both *EGFR* mutation and *EGFR* copy number in paired tumor specimens from patients with advanced NSCLC. We found that Scorpion-ARMS is more sensitive than direct sequencing for detection of *EGFR* mutations in small tumor specimens. Furthermore, we showed that *EGFR* mutation was significantly associated with *EGFR* amplification but not with FISH positivity. These observations warrant confirmation in further studies as well as exploration of the biological mechanisms of the relationship between *EGFR* mutation and *EGFR* amplification. The effects of *EGFR* mutation and *EGFR* copy number on clinical outcome in individuals with advanced NSCLC also warrant investigation in a prospective study.

Acknowledgments

We thank Tadao Uesugi, Mami Kitano, Erina Hatashita, and Yuki Yamada for technical assistance.

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doi: 10.1111/j.1349-7006.2008.00962.x

Jpn J Clin Oncol 2009;39(2)116–123
doi:10.1093/jjco/hyn135

Phase I Dose-escalation and Pharmacokinetic Trial of Lapatinib (GW572016), a Selective Oral Dual Inhibitor of ErbB-1 and -2 Tyrosine Kinases, in Japanese Patients with Solid Tumors

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Received August 24, 2008; accepted October 30, 2008; published online December 3, 2008

Objective: The Phase I dose-escalation study was conducted to evaluate the safety and pharmacokinetics of lapatinib (GW572016), a dual ErbB-1 and -2 inhibitor, in Japanese patients with solid tumors that generally express ErbB-1 and/or overexpress ErbB-2.
Methods: Patients received oral lapatinib once daily until disease progression or in an event of unacceptable toxicity.
Results: Twenty-four patients received lapatinib at dose levels of 900, 1200, 1600 and 1800 mg/day; six subjects enrolled to each dose level. The majority of drug-related adverse events was mild (Grade 1–2); the most common events were diarrhea (16 of 24; 67%), rash (13 of 24; 54%) and dry skin (8 of 24; 33%). No Grade 4 adverse event was observed. There were four Grade 3 drug-related adverse events in three patients (i.e. two events of diarrhea at 1600 and 1800 mg/day each and γ-glutamyl transpeptidase increase at 1800 mg/day). The maximum tolerated dose was 1800 mg/day. The pharmacokinetic profile of lapatinib in Japanese patients was comparable to that of western subjects.
Conclusions: Lapatinib was well tolerated at doses of 900–1600 mg/day in Japanese solid tumor patients. Overall, our findings were similar to those of overseas studies.

Key words: ErbB-1 – ErbB-2 – lapatinib – phase I – tyrosine kinase inhibitor

INTRODUCTION

Dysregulation of the human epidermal growth factor (ErbB) family of cell surface receptors has been noted in several solid tumors. Binding of extracellular ligand to ErbB receptors activates multiple intracellular signaling pathways that can promote tumor growth through processes, such as cell proliferation, differentiation and inhibition of apoptosis. ErbB-1 and ErbB-2 are implicated in the pathogenesis of several cancers (1), and their overexpression in epithelial tumors—including those of the lung, breast, head and neck, colon, stomach, ovary and prostate—often correlates with poor prognosis (2,3).

ErbB receptors present two rational targets for inhibition: blockade of the extracellular ligand-binding domain by monoclonal antibodies and inhibition of the intracellular tyrosine kinase domain by small molecules (4). Several anticancer agents target specific ErbB isoforms. For example, the small molecule tyrosine kinase inhibitors gefitinib (Iressa®) and erlotinib (Tarceva®) and the monoclonal antibody cetuximab (Erbitux®) all target ErbB-1 (5–7), and thus, they are indicated for the treatment of non-small cell lung cancer (NSCLC) and colorectal cancer (8,9). Furthermore, a monoclonal antibody directed against ErbB-2 (trastuzumab, Herceptin®) has been approved for patients with ErbB-2-overexpressing breast cancer (10). Sensitivity to some of these agents is strongly associated with the expression levels of ErbB-1 and -2 (2,3).

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Since it has been suggested that tumors with ErbB-1 expression and ErbB-2 overexpression are more aggressive than those without expression of the receptors (11–13), it has been proposed that dual inhibition of ErbB-1 and -2 could be a useful approach in patients with overexpression of these receptors. Lapatinib (GW572016) is a potent, orally active, small molecule dual inhibitor of ErbB-1 and -2. Lapatinib markedly reduces autophosphorylation of ErbB-1 and -2, and inhibits activation of Erk1/2 and AKT, the downstream effectors of cell proliferation and cell survival, respectively (14–17). Lapatinib inhibits tumor cell proliferation in various human tumor cell lines expressing ErbB-1 and overexpressing ErbB-2, as well as in tumor xenograft models (14–17).

Preclinical study of lapatinib revealed the agent to be well tolerated with an effective half-life of ~24 h, suggesting once-daily oral administration to be feasible (18). Clinical studies of the safety and efficacy of lapatinib in cancer patients are underway.

This was the first Japanese Phase I study of lapatinib in patients with solid tumors. This study was primarily designed to assess the safety of repeated oral doses of lapatinib in these patients and to investigate pharmacokinetics to see if they are comparable with those in western patients.

PATIENTS AND METHODS

Study Design

This was a non-randomized, open-label, multicenter, dose-escalation Phase I study conducted at two sites in Japan—Kinki University Hospital, Osaka and National Cancer Center Hospital East, Chiba.

The primary objectives were to assess the safety of repeated oral doses of lapatinib, to determine the maximum tolerated dose (MTD) in patients with solid tumors, to evaluate the pharmacokinetics (PK) of repeated oral doses of lapatinib and to compare the data from overseas studies and based on these data, to find the clinically recommended dose of lapatinib in Japanese patients enrolled in further studies.

Patient Eligibility

Adult patients aged 20–74 years with histologically or cytologically confirmed solid tumors that are generally known to express EGFR and/or overexpress ErbB-2 (including colorectal cancer, gastric cancer, NSCLC and breast cancer) were eligible for inclusion, provided that they had failed standard therapies or there were no other appropriate therapies available (19–40). Patients had to have normal function of major organs and adequate bone marrow, hepatic and renal functions defined as hemoglobin $\geq$9 g/dl, neutrophil count $\geq$1500/mm^3 and platelets $\geq$100 000/mm^3, AST and ALT $\leq$2.5 of upper limit of normal (ULN) and bilirubin $\leq$1.5 of ULN, and serum creatinine $\leq$1.5 of ULN, respectively. Left ventricular ejection fraction by echocardiography had to be $\geq$50% and in all patients an appropriate length of time since cessation of previous therapy was required (chemotherapy, radiotherapy, surgery or investigational products other than anticancer drugs, $\geq$4 weeks; nitrosourea compounds or mitomycin C, $\geq$6 weeks; biologic response modifiers or hormone therapy, $\geq$2 weeks). Patients were also to have an Eastern Cooperative Oncology Group performance status (PS) 0–2 and life expectancy $\geq$3 months after the start of lapatinib treatment.

Exclusion criteria were serious complications (Grade $\geq$3 according to the National Cancer Institute common toxicity criteria, NCI-CTC, version 2); pleural effusion, ascites and/or pericardial effusion requiring drainage by puncture, intracavital administration, or any other relevant treatment; systematic steroid use for $\geq$50 days or possible need for long-term use of systemic steroids; multiple active cancers; symptomatic brain metastases; malabsorption and/or total resection of the stomach or small intestine; corneal disorder; history of drug allergy; breast feeding; previous trastuzumab-induced impaired cardiac function; and previous acute pulmonary disorder or interstitial pneumonia induced by gefitinib.

All patients gave written informed consent before the start of study. The protocol was approved by the institutional review board of each study site. The study was conducted according to the World Medical Association Declaration of Helsinki (41) and Japanese good clinical practice guidelines (42).

Treatment

Based on the findings of overseas Phase I study (43), and in order to compare PK profiles with an overseas parallel Phase I study (44), patients were assigned to receive lapatinib 900, 1200 or 1600 mg/day for 21 consecutive days. Lapatinib was taken orally once daily with water after a light low-fat breakfast, except on Days 1 and 21 when it was administered in fasting state.

The dose levels started at 900 mg/day and increased to 1200 and 1600 mg/day, then increased by 200-mg increments until MTD was reached. MTD was defined as the dose at which dose-limiting toxicity (DLT), i.e. a drug-related adverse event of NCI-CTC Grade $\geq$3, occurred within 21 days after the initiation of dosage in two or more patients at each dose level with six subjects. When DLT was observed, the next dose for the patients was to be postponed, and could not restart until NCI-CTC grade became $\leq$2 within 14 days. In such cases, when NCI-CTC became Grade 2 or below, the dose was to be restarted at the previous dose level. When NCI-CTC did not reach Grade 2 or below after dose delays of 14 days, the treatment for the patients was to be discontinued. These dose delays and reductions were allowed to be performed only once.

Although appropriate supportive care and symptomatic treatment were allowed, prophylactic use (including

antiemetics) was not permitted between screening and Day 21 of the treatment period. Anticancer therapy of any kind, medications that may affect the absorption or metabolism of lapatinib, and other investigational drugs were prohibited throughout the study. Also, to prevent PK interactions, patients were instructed to avoid grapefruit, grapefruit juice and St John's Wort (*Hypericum perforatum*) throughout the study.

Safety Assessments

Assessments including clinical laboratory tests, vital signs, PS and body weight were performed at screening, at baseline (i.e. within 3 days before the first dose), on Days 7, 14 and 21, every 4 weeks thereafter, on cessation of treatment, and on the last day of observation (i.e. 28 days after the final dose or immediately before the start of next anticancer therapy). Chest X-ray, 12-lead electrocardiogram and echocardiography were performed at screening, once between Days 14 and 21, and on the last observation day. Toxicity was graded according to the NCI-CTC version 2.

Pharmacokinetic Analysis

For PK evaluation, 3-ml blood samples were collected at 1 h pre-dosing and at 1, 2, 3, 4, 6, 8, 10, 12 and 24 h after dosing on Days 1 and 21 and at pre-dosing on Days 7 and 14. Urine samples were collected before dosing on Day 1 and 0–24 h after dosing on Days 1 and 21.

Serum concentrations of lapatinib were measured by liquid chromatography tandem mass spectrometry with a lower limit of quantitation of 1 ng/ml.

The calculated PK parameters were maximum serum concentration (C_{max}), time to C_{max} (t_{max}), area under the plasma drug concentration–time curve from 0 to 24 h (AUC_{0-24}) and terminal half-life ($t_{1/2}$). Renal clearance was calculated from urine concentrations of lapatinib.

Efficacy Assessments

For efficacy assessment [i.e. tumor response as determined by X-ray, computed tomography (CT), magnetic resonance imaging (MRI) and/or other objective measurements according to the Response Evaluation Criteria in Solid Tumors (RECIST) guidelines (45)], evaluations were performed at screening (i.e. 4 weeks before the first dose of lapatinib), once during Days 14–21, every 4 weeks thereafter, and on the last day of observation. Target and non-target lesions were assessed in the same manner before and after dosing. Consistency of efficacy evaluation by the study investigators was assessed by extramural review committee.

RESULTS

Patients

Twenty-four patients were enrolled; all had received prior chemotherapy. Table 1 shows their baseline characteristics. The median age was 60 years (range, 37–73), and they had a median PS of 1. NSCLC was the main tumor type. Six patients at four dose levels, 900, 1200, 1600 and 1800 mg/day each, received lapatinib. Eight patients received lapatinib for >3 months and four for >6 months.

All patients completed the initial 21-day treatment period, although one of the patients had dose reduction (overall compliance, 90.5%) due to the onset of a Grade 3 drug-related adverse event (diarrhea) during this period. Four patients (three at 1200 mg dose level and one at 1600 mg dose level) withdrew from study due to disease progression and four (one each at 900 and 1600 mg dose level and two at 1800 mg dose level) were withdrawn at their own request. Mean durations of study treatment in the 900, 1200, 1600 and 1800 mg groups were 131, 68.2, 117 and 49.3 days, respectively. No patient withdrew due to adverse events.

Safety

All 24 patients were eligible for safety analysis. Table 2 lists the drug-related adverse events experienced by ≥20% of

Table 1. Baseline characteristics of patients

Characteristic	Dose (mg/day)				Total ($n = 24$)
	900 ($n = 6$)	1200 ($n = 6$)	1600 ($n = 6$)	1800 ($n = 6$)	
Sex					
Male	5	2	3	4	14
Female	1	4	3	2	10
Tumor type					
Non-small cell lung cancer	5	3	1	4	13
Adenocarcinoma	2	1	1	3	7
Squamous cell carcinoma	2	1	0	1	4
Other	1	1	0	0	2
Colorectal cancer	1	1	2	1	5
Breast cancer	0	0	2	0	2
Others	0	2	1	1	4
Performance status[a]					
0	2	1	2	3	8
1	4	5	3	3	15
2	0	0	1	0	1

[a]Eastern Cooperative Oncology Group performance status.