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Concurrent Chemoradiotherapy with Cisplatin and Vinorelbine for Stage III Non-small Cell Lung Cancer

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Introduction: Concurrent chemoradiotherapy with full doses of cisplatin-based chemotherapy is standard treatment for inoperable stage III non-small cell lung cancer (NSCLC). Although many platinum-based two drug combinations with third-generation agents are difficult to combine fully with thoracic radiotherapy (TRT), a phase I study reported a full dose of cisplatin (CDDP) plus 80% dose of vinorelbine (VNR) was successfully combined with concurrent TRT.

Methods: Between October 2000 and October 2004, 73 patients with inoperable stage III NSCLC treated with CDDP, VNR, and concurrent TRT were retrospectively analyzed. Patients were treated with CDDP 80 mg/m^2 on day 1 and VNR 20 mg/m^2 on days 1 and 8 every 4 weeks. Radiotherapy was administered concurrently in cycle 1. The total radiation dose was 60 Gy in 30 fractions. Common Terminology Criteria for Adverse Events version 3.0 were used to assess treatment-related adverse events.

Results: Median age was 63 years (40–78). Twenty-nine patients had adenocarcinoma, 63 were male, 47 ECOG PS 1, and 47 stage IIIB. Median chemotherapy cycle was 2.0. Objective response rate was 93% and median survival time was 21 months. Three-year overall survival rate was 33%. Infield control rate was 71%. The most common grade 3 or 4 adverse event was leukocytopenia (67%). Only 3 patients (4%) experienced grade 3 esophagitis. One patient died of radiation pneumonitis 87 days after completion of chemoradiotherapy.

Conclusions: Concurrent chemoradiotherapy with CDDP and VNR was highly active and well-tolerated. This regimen could be used as a control arm in future trial for stage III NSCLC.

Key Words: Concurrent chemoradiotherapy, Non-small cell lung cancer, Cisplatin, Vinorelbine.

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Lung cancer is the leading cause of cancer-related deaths throughout the world, including Japan.[1] Stage III inoperable non-small cell lung cancer (NSCLC) constitutes approx-

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imately 30% of all newly diagnosed cases of NSCLC.[2] Historically, patients with stage III NSCLC were treated with thoracic radiotherapy (TRT) alone. Nevertheless, the survival of patients treated with TRT alone was poor, with a 5-year survival rate of approximately 5%.[3] As the treatment option of chemoradiotherapy (CRT) has developed, the survival of patients with stage III NSCLC has improved, with 3-year survival of approximately 15–20% and median survival time (MST) of 15–20 months.[4,5] Several randomized trials have demonstrated that concurrent CRT using full dose of cisplatin-based chemotherapy improves long-term survival compared with sequential CRT.[6–9] Although two-drug combinations with cisplatin (CDDP) and third-generation agents including vinorelbine (VNR), docetaxel, paclitaxel, gemcitabine, and irinotecan are standard chemotherapy regimens for stage IV NSCLC,[10–12] it is difficult to deliver full doses of these regimens and concurrent TRT because of excessive toxicity.

Recently a phase I trial of CDDP, VNR, and concurrent RT was reported.[13] The recommended doses were CDDP 80 mg/m^2 on day 1 and VNR 20 mg/m^2 on days 1 and 8. Although this was a phase I study, an encouraging survival rate of 50% at 3 years was reported. On the basis of this result, we have treated inoperable stage III NSCLC patients with CDDP, VNR, and concurrent RT in clinical practice at the National Cancer Center Hospital East, Japan. Herein is our review of the efficacy and tolerability of CRT with CDDP and VNR.

MATERIALS AND METHODS

The objective of this retrospective analysis was to evaluate the efficacy and tolerability of concurrent CRT using CDDP and VNR.

Patient Selection

We reviewed consecutive 106 inoperable stage III NSCLC patients who were treated with CDDP, VNR, and concurrent TRT at the National Cancer Center Hospital East, Japan, between October 2000 and October 2004. Clinically apparent or histologically/cytologically proven N2/N3 disease or T4 otherwise pulmonary metastasis in the same lobe was considered "inoperable." Chest CT, abdominal CT/ultrasonography, bone scintigram or FDG-PET, and brain MRI/CT were performed in all patients. In general, lymph nodes that were larger than 1.0 cm in minor axis were considered as metastatic. Lymph nodes that were involved in multiple stations were considered 'clinically apparent N2/3.' To con-

firm N2 disease, which was detected in chest CT and considered 'not apparent,' FDG-PET and/or mediastinoscopy was performed. FDG-PET (or PET/CT) was performed in 18 patients. Mediastinoscopy was performed in ten patients. In addition, there were 5 histologically/cytologically confirmed N3 (supraclavicular lymph nodes) diseases. Thirty-three patients were excluded because they participated in a clinical trial that evaluated CDDP plus VNR followed by docetaxel,[14] therefore 73 patients were evaluated in the present analysis. Data of survival, recurrence, and treatments after failure were obtained from medical records. All patients were evaluated at weekly case conference in which radiation oncologists and medical oncologists who had special expertise in thoracic oncology made treatment decisions. Inclusion criteria for CRT in our institution were generally as follows; white blood cell count $>3.0 \times 10^9$/liter, platelet count $>10.0 \times 10^9$/liter, serum creatinine <1.5 mg/dl, total bilirubin <1.5 mg/dl, and transaminase less that twice the upper limit of the normal value. Exclusion criteria were pulmonary fibrosis identified by a chest x-ray, malignant pleural or pericardial effusion, and a concomitant serious illness, such as uncontrolled angina pectoris, myocardial infarction in the previous 3 months, heart failure, uncontrolled diabetes mellitus, *severe respiratory failure* and uncontrolled hypertension. All patients gave informed consent before CRT.

Chemotherapy

Chemotherapy consisted of CDDP (80 mg/m^2 on day 1) and VNR (20 mg/m^2 days on 1 and 8). Treatment cycles were repeated every 4 weeks with a maximum of 3 cycles administered. Cisplatin and VNR were administered by intravenous infusion. All patients received prophylactic antiemetic therapy consisting of 5-HT3 antagonist, metoclopramide, and dexamethasone. If a patient experienced excessive adverse events, dose reduction of both drugs was implemented during the subsequent treatment cycle. When leukocyte or platelet counts were inappropriate, or if infection developed at day 8, VNR was withheld.

Radiotherapy

TRT was administered concurrently in cycle 1. A CT-scan based treatment planning was used in all patients. The clinical target volume (CTV) for the primary tumor was defined as the gross tumor volume plus 0.5–0.8 cm margin taking account of subclinical extension. The CTV for metastatic lymph nodes were the same as the gross tumor volume for metastatic lymph nodes. Metastatic lymph nodes were defined as the lymph nodes that were larger than 1.0 cm in minor axis. Regional lymph nodes (mainly #3, #4, #7), excluding the contralateral hilar and supraclavicular lymph nodes, were included in the CTV for elective nodal irradiation. The planning target volume for the primary tumor, the metastatic lymph nodes, and regional lymph nodes was determined as CTVs plus setup margin (0.5 cm) and internal margins according to the respiratory motion on fluoroscopy (circumferential 0.5 cm, cranial 0.5 cm, and caudal 1.0–1.5 cm). Lung heterogeneity corrections were not used, and the doses were prescribed to the center of planning target volume. Principally, the initial radiation field was planned not to

exceed 50% of ipsilateral lung volume on chest radiograph, or since August 2003, V20 of the normal lung (the percent volume of normal lung receiving 20 Gy or more) was planned not to exceed 35%. The total radiotherapy dose was 60 Gy in 30 fractions (5 fractions per week) delivered over 6 weeks. Radiation therapy was delivered with megavoltage equipment (6 mV) using parallel opposed fields up to 40 Gy in 20 fractions including primary tumor, the metastatic lymph nodes, and the regional lymph nodes. A booster dose of 20 Gy in 10 fractions was given to the primary tumor and the metastatic lymph nodes according to the CT obtained after initial 40 Gy radiation, using opposed oblique fields to avoid excessive dose to the spinal cord.

Evaluation of Efficacy and Adverse Events

Overall survival was defined as time from start of chemoradiotherapy to death of any cause. Progression-free survival was defined as time from start of chemoradiotherapy to the first documented disease progression or death. Disease progression was subdivided into infield relapse or not. Chest CT was used to asses if the relapse was within the initial radiation field. Response Evaluation Criteria in Solid Tumor criteria were used to assess the best tumor response. Chest CT was reviewed independently by a radiologist. The response rate was calculated as the total percentage of patients with a complete or partial response. In principle, the chest CT was taken 2 and 4 months after starting chemoradiotherapy and as needed to evaluate the response and toxicity. Treatment-related adverse events were evaluated using the Common Terminology Criteria for Adverse Events Version 3.0. Late toxicities were scored according to the European Organization for Research and Treatment of Cancer/Radiation Therapy Oncology Group late radiation morbidity scoring scheme.

Statistical Analyses

Multivariate analyses were performed using Cox regression models. Expected prognostic factors included age (<70 years versus >70), gender (male versus female), Eastern Cooperative Oncology Group (ECOG) performance status (0 versus 1), clinical stage (IIIA versus IIIB), smoking history (<30 pack-year versus >30), histology (adenocarcinoma versus others), tumor size (<5 cm versus >5 cm), stage (IIIA versus IIIB), and weight loss ($<5\%$ versus $>5\%$). Kaplan–Meier methods were used to graphically describe the distribution of survival. All statistical analyses were performed using SPSS II for Windows version 11.0.1J.

RESULTS

Patients' characteristics are shown in Table 1. Median number of chemotherapy cycles were 2.0 (mean 2.4, ranges 1–3). Dose reduction of chemotherapy was implemented in 11 patients mainly due to grade 4 leukocytopenia. Two patients did not receive full dose of radiotherapy. In one patient, radiotherapy was discontinued at the dose of 40 Gy because the tumor was located nearby the spinal cord, and in the other patient because of declined PS.

All 73 patients were assessable for survival, time to progression, response rate, and adverse events. No patient achieved complete response. Partial response, stable disease,

TABLE 1. Patient Characteristics

	Patients (n = 73)	
	No.	%
Age		
Median (range) (yr)	63 (40–78)	
<70 yr	48	66
≥70 yr	25	34
Gender		
Female	10	14
Male	63	86
Histological diagnosis		
Adenocarcinoma	29	40
Squamous cell carcinoma	28	38
Others	16	22
Tumor size		
Median (range) (cm)	5.4 (1.5–12.0)	
<5 cm	33	45
≥5 cm	40	55
ECOG performance status		
0	26	36
1	47	64
Smoking history		
Never smoker	5	7
<30 pack-yr	11	15
≥30 pack-yr	57	78
Stage		
IIIA	26	36
T3N1	3	4
N2	23	32
IIIB	47	64
T4[a]	40	55
N3	12	16
Body weight loss (recent 6 mo)		
<5%	58	79
≥5%	15	21

[a] Six were T4N0, 3 were T4N1, and 5 were T4N3.

TABLE 2. Overall Objective Response

	Number	%
Number of patients evaluated	73	
Complete response (CR)	0	0
Partial response (PR)	68	93.2
Stable disease (SD)	5	7.8
Progressive disease (PD)	0	0
Response rate (95% CI)		93.2 (87.2–99.1)%

CI, confidence interval.

and progressive disease were observed in 68, 5, and 0 patient, respectively (Table 2). The response rate was 93.2% (95% confidence interval; 87.2–99.1%). Median progression free survival time was 12 months and median overall survival time was 21 months with median follow-up of 35 months (ranges 23.7–61.2). Two- and 3-year survival rate was 44 and 33%, respectively. The Kaplan–Meier plots of overall survival are shown in Figure 1; Figure 2 shows progression-free
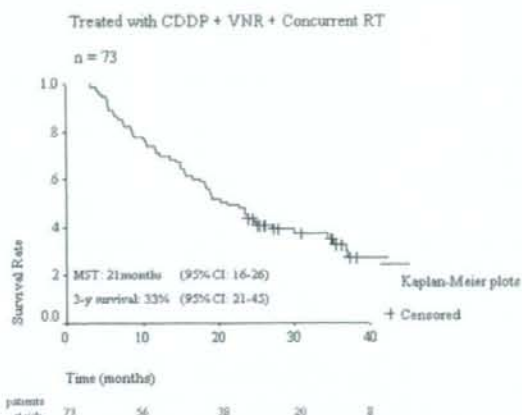

FIGURE 1. Overall survival of patients treated with CDDP + VNR + concurrent RT. CDDP, cisplatin; VNR, vinorelbine; RT, radiotherapy; MST, median survival time; 3-year survival, survival rate at 3 years.
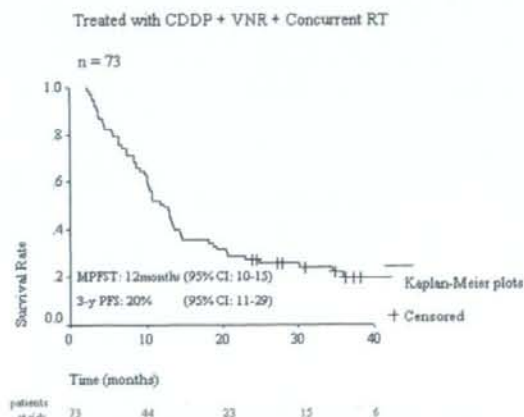

FIGURE 2. Progression-free survival of patients treated with CDDP + VNR + concurrent RT. CDDP, cisplatin; VNR, vinorelbine; RT, radiotherapy; MPFST, median progression-free survival time; 3-year survival, progression-free survival rate at 3 years.

survival. Multivariate analysis showed that no variables significantly affected the overall survival (Table 3).

There were 46 disease relapses and 50 deaths. Infield relapses were observed in 21 patients (11 without and 10 with relapse outside of the radiation fields); therefore infield control rate was 71%. Distant metastases were the first sites of the failure in 35 patients; brain (n = 16), bone (n = 10), adrenal gland (n = 5), liver (n = 3), and lung (n = 16). Seventeen patients received docetaxel and 12 received gefitinib as second line treatment. None responded to docetaxel and two patients (16%) responded to gefitinib (and 1 achieved partial response).

TABLE 3. Prognostic Factors Treated with CDDP + VNR + Concurrent TRT ($n = 73$)

Parameter	Hazard Ratio	95% CI	p
Age (<70 yr vs. ≥70)	1.787	0.941–3.394	0.076
Gender (male vs. female)	1.364	0.490–3.799	0.553
PS (0 vs. 1)	0.818	0.435–1.537	0.533
Clinical Stage (IIIA vs. IIIB)	1.109	0.588–2.093	0.749
Smoking (<30 pack-yr vs. ≥30)	0.698	0.321–1.519	0.365
Tumor size (< 5 cm vs. ≥5)	0.862	0.473–1.569	0.626
Histology (Ad vs. others)	1.565	0.766–3.198	0.219
Body weight loss (<5% vs. ≥5)	1.567	0.786–3.125	0.202

CI, confidence interval; Ad, adenocarcinoma.

The incidence of treatment-related adverse events is listed in Table 4. The most common grade 3 or 4 adverse event was leukocytopenia (67%). Grade 3 or 4 neutropenia was observed in 38 patients (52%). Grade 3 or 4 thrombocytopenia was not observed; grade 3 or 4 anemia occurred in 17 patients (23%). Only 3 patients (4%) experienced grade 3 esophagitis related to radiotherapy. Five patients (7%) developed grade 3 or 4 pneumonitis and one of them died of respiratory failure 87 days after completion of chemoradiotherapy. The autopsy revealed diffuse alveolar damage compatible with radiation pneumonitis and fibrosis. None of the 5 patients with grade 3 or 4 pneumonitis received second line chemotherapy. Another patient of them developed grade 3 pulmonary fibrosis, but no other severe late radiation morbidity was observed.

DISCUSSION

Chemoradiotherapy is standard treatment for patients with inoperable stage III NSCLC. Several trials indicate that

TABLE 4. Grade 3 or 4 Treatment-Related Adverse Events (NCI-CTC vs. 3.0, $n = 73$)

Adverse Event	Grade 3 (%)	Grade 4 (%)
Leukocytes	32	36
Neutrophiles/granulocytes	25	27
Hemoglobin	22	1
Platelets	1	0
Febrile neutropenia	14	0
Infection with grade 3 or 4 neutropenia	1	0
Infection without neutropenia	10	0
Pneumonitis/pulmonary infiltrates	5	1[a]
Radiation esophagitis	4	0
Radiation dermatitis	0	0
Anorexia	16	0
Nausea	8	0
Vomiting	5	0
Diarrhea	1	0
Creatinine	0	0
Supraventricular arrhythmia (atrial fibrillation)	1	0

[a] One patient died from radiation pneumonitis 87 d after completion of chemoradiotherapy.

concurrent CRT improves long-term survival compared with sequential CRT.[6–9] Nevertheless, the optimal regimen and dose of chemotherapy has not been determined yet. The efficacy of chemoradiotherapy with CDDP and vinca alkaloids or etoposide has been reported, and CDDP plus vindesine with or without mitomycin has been one of the standard chemotherapy regimens.[6,15–17]

VNR is a newer semi-synthetic vinca alkaloid and more active than vindesine against metastatic NSCLC.[18] Zatloukal et al.[8] reported the efficacy of CRT with CDDP and VNR in a randomized phase II trial, which randomized concurrent CRT or sequential. Concurrent arm was favored in overall survival (MST was 16.6 months in the concurrent arm and 12.9 months in the sequential arm). Vokes et al.[19] also reported the efficacy of CRT with CDDP and VNR in randomized phase II trial, which randomized 3 CDDP-based combination chemotherapies with third-generation agents. In this series, MST of all patients were 17 months and 3 year survival of VNR arm was 23%. With these results, concurrent CRT with CDDP and VNR could be considered one of the new standard regimens for stage III NSCLC, although the employed VNR doses in each phase II study were 12.5 mg/m^2 and 15 mg/m^2. Standard doses of CDDP plus VNR for metastatic NSCLC are 80 mg/m^2 of CDDP and 25 mg/m^2 of VNR. The doses of 20 mg/m^2, employed in the present study, are close to the standard. Moreover, 20 mg/m^2 of VNR alone has reported to be active in advanced NSCLC, with response rate of 21.7%.[20]

Results of the present study were encouraging, demonstrating MST of 21 months and a 3-year survival rate of 33%. Our study confirmed clinical usefulness of combination chemotherapy with CDDP, VNR, and simultaneous TRT.

The most common treatment-related adverse events were hematological (grade 3 or 4 leukocytopenia in 67%, neutropenia in 52%, and anemia in 23%), and these were well tolerated. There were 5 patients (7%) who developed grade 3 or more pneumonitis and only one patient (2%) died of radiation pneumonitis. The incidence and mortality of radiation pneumonitis was comparable with other reports.[6,8,9,19,21–24] Recently we have evaluated dose volume histogram and plan V20 not to exceed 35% in CRT, which may contribute to reducing severe radiation pneumonitis.

Low incidence of severe radiation-related esophagitis in our study deserves special mention. In the present study grade 3 esophagitis was developed in only 3 patients (4%), which is lower than other studies of concurrent chemoradiotherapy where radiation-related esophagitis was reported to be in the range of 12–46%,[21–23] with the exception of one study using CDDP, vindesine (VDS), and mitomycin.[6] In this report, the incidence of grade 3 or more radiation-related esophagitis was only 3%. The cause of this difference is still unknown; however, low incidence of esophagitis may correlate with the use of vinca alkaloids and Japanese studies. Further examination is warranted. We believe that highly conformal therapy could reduce the rate of esophagitis. Overall, chemoradiotherapy with CDDP and VNR were well tolerated.

Although the collection of toxicity data retrospectively is of concern, most patients were treated as inpatient through-

out the treatment course, and toxicity data were recorded on medical records in detail. It should be confirmed by a prospective study.

Taxanes are also investigated widely in patient with unresectable stage III NSCLC. Weekly administration with carboplatin (CBDCA) plus paclitaxel (PTX) and concurrent RT was reported in multiinstitutional phase II study. Reported MST was promising, with 20.5 months.[25] Nevertheless, recently reported phase III trial compared induction chemotherapy plus CRT with CRT alone, which employed weekly CBDCA and PTX, showed disappointing results, with MST of 14 months and 12 months, respectively.[26] The authors concluded that the routine use of weekly CBDCA and PTX with simultaneous TRT should be re-examined. Chemotherapy with docetaxel (DOC) plus CDDP and concurrent TRT was also reported in a phase I/II study.[21] The result was promising, with MST of 23 months, and phase III trial comparing DOC and CDDP to CDDP, VDS, and mitomycin is currently underway.

Local recurrence was observed in 21 patients (29%), and the brain was also a major site of treatment failure (16 patients, 22%). These results are comparable to the literature.[21] On the basis of these observations, other radiation approaches such as hyperfractionated radiotherapy or high-dose thoracic radiation to improve local control should be considered.[27–31] Moreover, whether prophylactic cranial irradiation reduces the incidence of brain metastases should be confirmed.

Advanced age did not correlate with worse prognosis and it is compatible with literature.[32] Gender, tumor size, body weight loss, smoking status did not significantly correlate with shorter overall survival, and it may be due to the small sample size of our study.

We excluded 33 patients who participated in the trial evaluated consolidation docetaxel after concurrent CRT with CDDP and VNR.[14] Sekine and colleagues reported that majority of patients could not continue with consolidation docetaxel after concurrent CRT with CDDP and VNR because of pulmonary toxicity. Although consolidation therapy using docetaxel seems to be highly effective in SWOG phase II study,[33] randomized phase III trial failed to demonstrate that addition of consolidation docetaxel improves survival.[34]

Two patients did not receive full dose of radiotherapy. Nevertheless, these two patients were treated initially with curative intent. Therefore we included these two patients in this analysis. Moreover, exclusion of these two patients did not alter the results (data not shown).

In conclusion, chemoradiotherapy with CDDP and VNR was promising and well tolerated. This regimen could be used as a control arm in future trial for stage III NSCLC.

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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 naïve 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).

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 ($\geqslant$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.
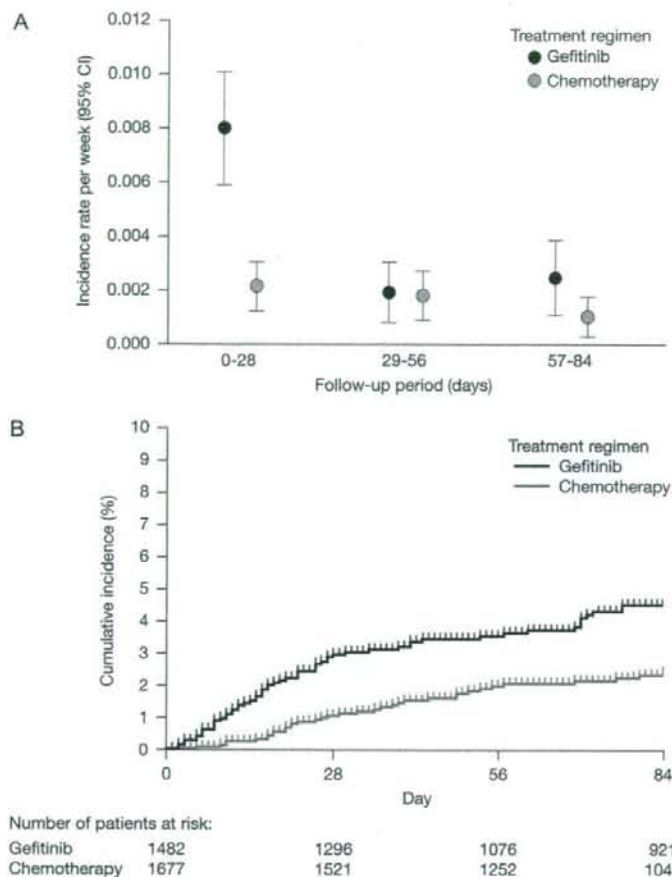
Figure 1. (*A*) Incidence rates of acute interstitial lung disease (ILD) in Japanese patients with non–small cell lung cancer for gefitinib and chemotherapy cohorts by 4-week period after treatment initiation. (*B*) Kaplan-Meier curves of risk of ILD to 12 weeks for the observed cohorts. CI = confidence 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 ($\leq 50\%$) or extensive areas adherent to pleura ($\geq 50\%$). 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,
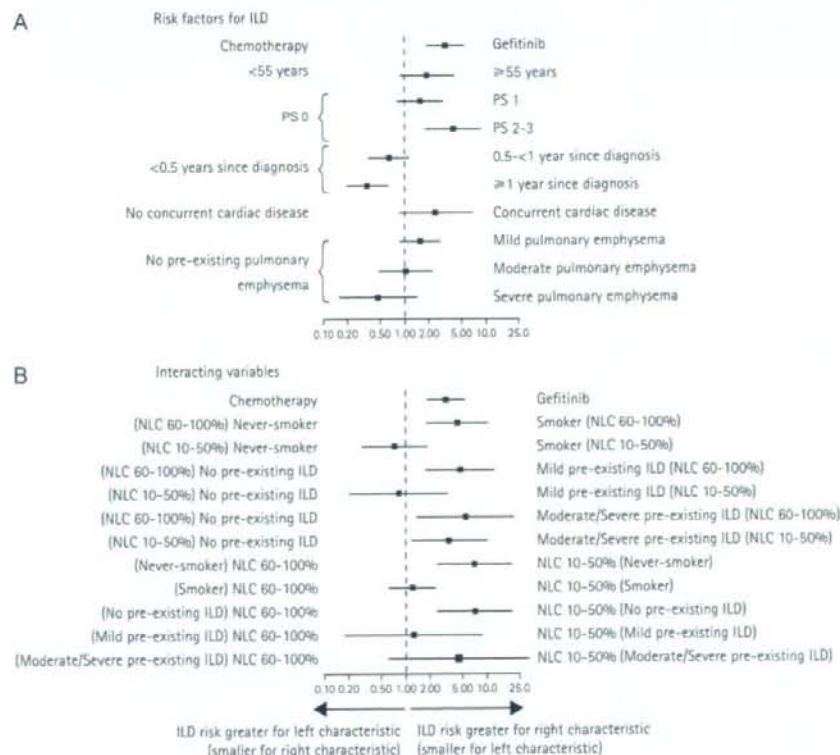
Figure 2. Adjusted odds ratios for risk factors for acute interstitial lung disease (ILD) in Japanese patients with non–small cell lung cancer from final logistic model. NLC = normal lung coverage (extent of normal lung on computed tomography scan); PS = World Health Organization performance status.
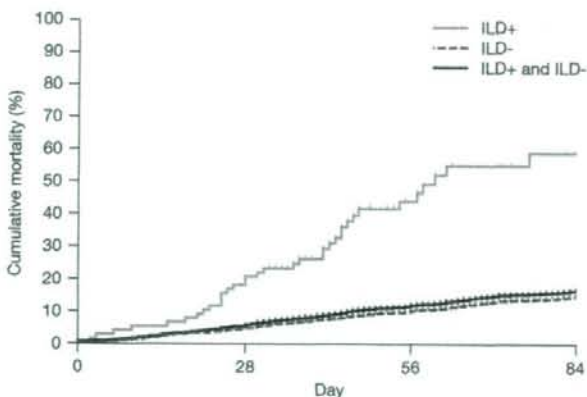
Figure 3. Kaplan-Meier curves showing risk of death to 12 weeks in the gefitinib cohort overall and subdivided into those that developed interstitial lung disease (ILD+) and those who did not (ILD−).

Number of patients at risk:

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

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.

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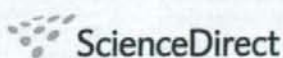
Mutational status of *EGFR* and *KIT* in thymoma and thymic carcinoma

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Summary   This study was conducted to evaluate the prevalence of *EGFR* and *KIT* mutations in thymomas and thymic carcinomas as a means of exploring the potential for molecularly targeted therapy with tyrosine kinase inhibitors. Genomic DNA was isolated from 41 paraffin-embedded tumor samples obtained from 24 thymomas and 17 thymic carcinomas. *EGFR* exons 18, 19, and 21, and *KIT* exons 9, 11, 13, and 17, were analyzed for mutations by PCR and direct sequencing. Protein expression of EGFR and KIT was evaluated immunohistochemically. *EGFR* mutations were detected in 2 of 20 thymomas, but not in any of the thymic carcinomas. All of the *EGFR* mutations detected were missense mutations (L858R and G863D) in exon 21. EGFR protein was expressed in 71% of the thymomas and 53% of the thymic carcinomas. The mutational analysis of *KIT* revealed only a missense mutation (L576P) in exon 11 of one thymic carcinoma. KIT protein was expressed in 88% of the thymic carcinomas and 0% of the thymomas. The results of this study indicate that *EGFR* and *KIT* mutations in thymomas and thymic carcinomas are rare, but that many of the tumors express EGFR or KIT protein.
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1. Introduction

Thymic epithelial tumors are uncommon neoplasms and there are two major histological types: thymoma and thymic

carcinoma [1]. Surgical resection is the preferred treatment option for all subtypes of thymoma and thymic carcinoma. However, thymic carcinomas and some thymomas tend to behave in a malignant manner clinically, and in many cases dissemination or distant metastasis has already occurred at presentation. Patients with metastatic or unresectable tumors are candidates for systemic chemotherapy, but no standard chemotherapy has been established because of the rarity of both tumors [2—5], and alternative therapeutic molecular targets are needed.

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Receptor tyrosine kinases such as epidermal growth factor receptor (EGFR) and KIT, contribute to a number of processes related to the survival and growth activity of many solid tumors, making them promising targets for cancer therapy [6–8]. Recent studies have shown that the presence of kinase domain mutations in the EGFR gene in non-small cell lung cancer (NSCLC) tissue predicts a significant clinical response to small-molecule tyrosine kinase inhibitors (TKIs) of EGFR, such as gefitinib and erlotinib [9], and it is widely known that there is an association between exon 11 mutations of the KIT gene in gastrointestinal stromal tumors (GISTs) and greater responsiveness to imatinib as a small-molecule TKI of KIT [10].

Several immunohistochemical studies have shown overexpression of EGFR protein in both thymoma and thymic carcinoma [11,12], and in thymic carcinoma, immunohistochemical studies have shown a high frequency of KIT overexpression but that thymomas express hardly any KIT [13,14]. Two interesting cases have recently been reported. One was a case of thymic carcinoma with an activating *KIT* mutation that responded to imatinib, reported by Strobel et al. [15], and the other was a case of thymic carcinoma with *EGFR* mutations that was responsive to gefitinib, reported by Yamaguchi et al. [16]. However, because of the rarity of these tumors, information on the mutational status of *EGFR* and *KIT* in thymomas and thymic carcinomas has been limited to only a few reports, and the prevalence of *EGFR* and *KIT* mutations remains unknown.

In this study, we investigated the status of *EGFR* and *KIT* mutations in thymoma and thymic carcinoma patients to explore the potential for molecularly targeted therapy with TKIs. We also investigated the relation between protein expression assessed by immunohistochemistry and the mutational status of *EGFR* and *KIT*.

2. Patients and methods

2.1. Patients

The tumor samples used in this study were obtained from paraffin-embedded surgical specimens from 41 cases of thymoma or thymic carcinoma treated surgically at the National Cancer Center Hospital East between 1993 and 2005. All samples were reviewed to confirm the diagnosis of thymoma or thymic carcinoma. The clinical data of all patients was collected from their medical records. This study was approved by the Institutional Review Board of our institution.

The characteristics of all of the patients are listed in Table 1. Patient age ranged from 21 to 77 years, and their median age was 61 years. The specimens used were from 24 thymomas and 17 thymic carcinomas. According to the World Health Organization (WHO) classification of thymic epithelial tumors, the histological subtype of the thymomas was type A in 7 cases, type AB in 7 cases, type B1 in 6 cases, and type B2 in 4 cases. The histological subtype of the thymic carcinomas was squamous cell carcinoma in 14 cases, and adenocarcinoma, adenosquamous carcinoma, and non-specified in 1 case each. According to the system described by Masaoka et al. [17], the clinical stage was stage I in 15 patients, stage II in 8 patients, stage III in 9 patients, stage

Table 1 Patient characteristics

	Patients (n = 41)
Age, years	
Median	61
Range	21–77
Gender	
Female	20
Male	21
Histology	
Thymoma	24
Thymic carcinoma	17
Stage	
I	15
II	8
III	9
IVa	1
IVb	8
Surgical procedure	
Total resection	36
Partial resection	5
Smoking history	
Never	19
Former	11
Current	11

IVa in 1 patient, and stage IVb in 8 patients. All patients had undergone total resection (n = 36) or partial resection (n = 5) after obtaining their informed consent in accordance with institutional guidelines.

2.2. Mutational analysis of *EGFR* and *KIT*

Tumor genomic DNA was isolated from paraffin-embedded samples of a total of 41 tumors, 24 thymomas and 17 thymic carcinomas. To ensure that tumor-cell-rich areas of tissues were isolated, hematoxylin and eosin stained slides were prepared from each selected paraffin-embedded block. Polymerase chain reaction (PCR) was performed to amplify exons 18, 19, and 21 of *EGFR* and exons 9, 11, 13, and 17 of *KIT* by using previously described primers [9,18], and the PCR products were directly sequenced with an ABI 3100 DNA Sequencer (Applied Biosystems, Foster City, CA, USA). All sequencing reactions were performed in both forward and reverse directions. A series of mutational analyses was performed at Mitsubishi Chemical Safety Institute Ltd.

2.3. Immunohistochemistry

Protein expression of EGFR and KIT was evaluated immunohistochemically in representative paraffin-embedded sections. EGFR staining was performed by using the DAKO (Carpinteria, CA, USA) pharmDX kit for EGFR according to the manufacturer's instructions, and immunostaining for KIT was performed by using a polyclonal rabbit antibody (A 4502; Dako, Glostrup, Denmark) according to the manufacturer's instructions. Staining of both markers was considered posi-

tive if more than 50% of the tumor cells stained. All slides were examined and scored independently by two observers (G.I. and K.Y.).

2.4. Statistical analysis

The variables measured in the study were tested for associations by Fisher's exact test. P values <0.05 were considered statistically significant.

3. Results

3.1. EGFR analysis of thymomas and thymic carcinomas

Sequencing of the EGFR tyrosine kinase domain encoded by exons 18, 19, and 21 was successful in 29 of the 41 tumors (Table 2). EGFR mutations were detected in 2 of the 20 thymomas, but direct sequencing showed no evidence of mutations in any of the 9 thymic carcinomas. All of the EGFR mutations detected were missense mutations in exon 21 (L858R or G863D), and no mutations were detected in exons 18 and 19. Examination of 21 thymomas and 17 thymic carcinomas for EGFR protein expression by immunohistochemistry revealed EGFR expression in 15 (71%) of the 21 thymomas and 9 (53%) of the 17 thymic carcinomas. The difference in EGFR expression between the thymomas and thymic carcinomas was not significant ($P = 0.31$).

3.2. KIT analysis of thymomas and thymic carcinomas

It was possible to analyze the KIT mutation status of 22 thymomas and 11 thymic carcinomas by direct sequencing (Table 3). A missense mutation in exon 11 (L576P) was found in only one thymic carcinoma, and direct sequencing of KIT exons 9, 13, and 17 revealed no mutations in any of the tumors analyzed. Immunohistochemistry showed KIT protein expression in 15 (88%) of the 17 thymic carcinomas, but no KIT expression in any of the 24 thymomas ($P < 0.0001$).

Table 4 summarizes the data of all patients whose tumors were positive for EGFR or KIT mutations. Exon 21 mutations in the EGFR gene were found in two thymomas (Fig. 1A and B), and an exon 11 mutation was identified in the KIT gene of 1 thymic carcinoma (Fig. 1C). Because these muta-

Table 2 EGFR status of thymomas and thymic carcinomas

EGFR mutation	Thymoma (n = 20)	Thymic carcinoma (n = 9)	
Exon 18	0	0	
Exon 19	0	0	
Exon 21	2	0	
No mutation	18	9	
EGFR expression	Thymoma (n = 21)	Thymic carcinoma (n = 17)	P
Positive	15 (71%)	9 (53%)	0.31

Table 3 KIT status of thymomas and thymic carcinomas

KIT mutation	Thymoma (n = 22)	Thymic carcinoma (n = 11)	
Exon 9	0	0	
Exon 11	0	1	
Exon 13	0	0	
Exon 17	0	0	
No mutation	22	10	
KIT expression	Thymoma (n = 24)	Thymic carcinoma (n = 17)	P
Positive	0 (0%)	15 (88%)	< 0.0001

tions were not detected in the normal lung tissues from the same patients, they were considered to be somatic mutations. Both patients whose tumors were positive for EGFR mutation were never smokers. All three patients had undergone surgical resection, and they are currently alive and relapse-free.

4. Discussion

In this study, EGFR mutations were observed in the DNA sequences of 2 thymomas of 29 tumors analyzed, and analysis of the KIT mutation status of 22 thymomas and 11 thymic carcinomas by direct sequencing revealed a missense mutation in exon 11 in only 1 thymic carcinoma. By contrast, 71% of the thymomas and 53% of the thymic carcinomas expressed EGFR protein, and overexpression of KIT was observed in 88% of the thymic carcinomas and 0% of the thymomas. The results show that the EGFR and KIT protein expression in the thymomas and thymic carcinomas was not associated with EGFR or KIT mutations.

A review of the medical literature retrieved reports of two studies that investigated EGFR mutations in thymomas or thymic carcinomas [19,20] and of one study that tested thymic carcinomas for KIT mutations [13]. Suzuki et al. reported that direct sequencing did not reveal any EGFR missense mutations in a total of 38 thymoma samples obtained from Japanese patients [19]. Meister et al. reported detecting no mutations in the tyrosine kinase domain of EGFR in 20 DNA samples from 17 thymomas and 3 thymic carcinomas analyzed by direct sequencing [20]. Pan et al. performed a mutation analysis of KIT by direct DNA sequencing in 21 thymic carcinomas, but found none [13]. To date, EGFR mutations (double missense mutations: G719A in exon 18 and L858R in exon 21) have been reported in one case of thymic carcinoma [16], and a KIT mutation (V560del in exon 11) in one case of thymic carcinoma [15]. The results of our study and review of the literature suggest that EGFR or KIT mutations are rare in thymomas and thymic carcinomas but that expression of EGFR and KIT is frequently present. Mutations that activate receptor tyrosine kinases contribute to the development of human carcinomas, and the activation of a mutation in the KIT gene is thought to be the most important factor in the pathogenesis of GISTs [7,8]. However, we speculate that EGFR or KIT mutations may not be implicated in the carcinogenesis of thymomas and thymic