Table 6. Cox Model to Compare Outcomes by Polymorphism

Outcome by Polymorphism	Analyses			
	Comparison	HR	95% CI	P
ABCB1 3425				
Overall survival	Com *v* Het/Var (CC *v* CT/TT)	1 09	0 71 to 1.67	69
PFS		1 04	0 70 to 1 56	82
Response		0 97	0 39 to 2 38	1.00
Neutropenia		0.54	0 22 to 1 30	19
CYP2C8 R139K				
Overall survival	Com *v* Het/Var (GG *v* GA/AA)	1 09	0 61 to 1 96	76
PFS		1 12	0 63 to 2 00	69
Response		1.92	0 46 to 11.11	51
Neutropenia		1 30	0 35 to 5 00	87
*CYP3A4**1B				
Overall survival	Com *v* Het/Var (AA *v* AG/GG)	0 74	0 32 to 1 72	.48
PFS		0 36	0 14 to 0.94	.04
Response		0 63	0.10 to 4.76	.84
Neutropenia		0 44	0 04 to 2 94	58
*CYP3A5**3C				
Overall survival	Com/Het *v* Var (AA/AG *v* GG)	1 64	0 95 to 2 86	07
PFS		1 56	0 93 to 2 63	09
Response		1 61	0 53 to 4 76	47
Neutropenia		1 30	0 44 to 3 85	78
ERCC1 (118)				
Overall survival	TT *v* TC/CC	1 20	0 74 to 1 96	45
PFS		1 11	0.69 to 1 82	.65
Response		1 45	0 48 to 4.17	61
Neutropenia		0 57	0 20 to 1.61	35
ERCC2 K751Q				
Overall survival	Com *v* Het/Var (AA *v* AC/CC)	0 97	0 63 to 1 49	89
PFS		0 85	0 55 to 1 30	45
Response		0 33	0 13 to 0 83	02
Neutropenia		0 75	0 30 to 1 85	63
nr1I2-206 del				
Overall survival	Com *v* Het/Var 206 deletion	0.82	0 53 to 1 25	.35
PFS		0.93	0.63 to 1.39	.75
Response		0 82	0.34 to 2 00	.77
Neutropenia		0 88	0 37 to 2 08	.90

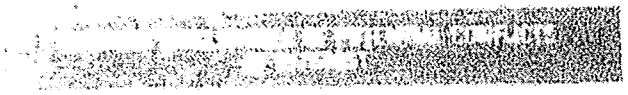
Abbreviations: HR, hazard ratio; PFS, progression-free survival; Com, common allele; Het, heterozygous allele; Var, variant allele

neutropenia ($P = .19$) for patients who harbored the ABCB1 3435 common allele.

The *ERCC2* gene, also known as xeroderma pigmentosum complementation group D, encodes a DNA helicase which complexes with TFIIH, a transcription factor essential for replication and nucleotide excision repair.[31] Several nonsynonymous SNPs have been described in this gene, including an Asp→Asn (G→A) at codon 312 in exon 10 and a Lys→Gln (A→C) at codon 751 in exon 23 and are likely in linkage disequilibrium with each other.[32,33] The functional consequences of these SNPs are still in contention, and the majority of studies indicate that variants in these alleles result in reduced DNA repair capacity.[34-41] Additionally, most studies indicate that *ERCC2* variants confer an increased risk of lung cancer.[32,34,35,42-48] In this study, 51% of patients (ie, 37 of 72 patients) from the US were homozygous wild type for the common (A) allele. These patients were significantly less likely to respond to treatment compared with US patients who had one or more variant alleles (A/C or C/C). However, no differences in overall survival were observed on the basis of *ERCC2* K751Q allele frequencies. In addition, this allele cannot account for the improved survival experienced by Japanese patients, as they uniformly harbored the common A/A genotype (and only one patient harbored A/C). The *ERCC1* 118 C→T SNP does not result in an amino acid substitution, although studies have nevertheless identified associations with patient outcome in various tumor types.[49] It has been suggested that this variant may modulate *ERCC1* mRNA and protein expression and/or may be in linkage disequilibrium with other functional SNPs.[14,50,51] However, three reports in NSCLC found no associations between the *ERCC1* 118 and patient outcome.[52-54] Here, we found a highly significant divergence in allele frequency between Japanese and US patients ($P < .0001$); however, no impact on patient outcome was observed.

In summary, the results of cancer clinical trials to test the same regimen may differ for a variety of reasons, including differences related to ethnicity. FACS, LC00-03, and S0003 were prospectively designed to facilitate a comparison of patient outcomes and pharmacogenomic results, in a setting where joint clinical trials sponsored by the US National Cancer Institute were not possible. Our

results suggest that global clinical trials (ie, those conducted internationally) should be carefully designed and conducted to account for potential genetic differences in the patient populations studied. This common-arm approach provides a model for the prospective study of population-related pharmacogenomics in which ethnic differences in antineoplastic drug disposition are anticipated.



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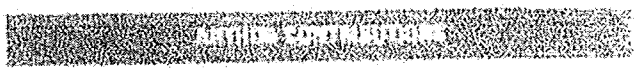

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Efficacy and Safety of Erlotinib Monotherapy for Japanese Patients with Advanced Non-small Cell Lung Cancer

A Phase II Study

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Introduction: The aim of this study was to evaluate the efficacy and safety of Erlotinib in Japanese patients with previously treated non-small cell lung cancer (NSCLC). Available tumor biopsy samples were analyzed to examine relationships between biomarkers and clinical outcome.

Methods: This open-label phase II trial enrolled stage III/IV NSCLC patients who had progressive disease after at least one prior platinum-based chemotherapy regimen. Erlotinib was administered at a dose of 150 mg/d orally until disease progression or intolerable toxicity. Analysis of epidermal growth factor receptor gene mutations in exon 18–21 by direct sequencing was performed in tumor tissue specimens obtained at the first diagnosis.

Results: Sixty-two patients were enrolled and 60 patients were evaluable for efficacy. Objective response rate and disease control rate were 28.3% and 50.0%; median time to progression and overall survival were 77 days and 14.7 months, respectively. In logistic regression analysis, only smoking history was proved to be a statistically significant predictive factor for response (odds ratio: 0.06, $p < 0.001$). Only 7 patients had samples available for mutation analysis. Three patients who had deletion mutations on exon 19 (del E746-A750 or del S752-I759) exhibited objective response. Common toxicities were rash (98%), dry skin (81%), and diarrhea (74%). Discontinuation due to adverse events occurred in 11 patients (18%). Four patients (6%) experienced interstitial lung disease-like events, one of whom died.

Conclusion: Erlotinib is efficacious in Japanese patients with previously treated NSCLC. The toxicity profile was similar to that in Western patients, except for a somewhat higher incidence of skin disorders and interstitial lung disease. Further studies are needed to determine the relationship between epidermal growth factor receptor mutations and outcomes with Erlotinib in Japanese patients.

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Lung cancer affects approximately 1.2 million people annually, and is the leading cause of cancer death in the world.[1] More than 80% of affected patients are diagnosed with non-small cell lung cancer (NSCLC). The standard first-line treatment for metastatic NSCLC is a combination of platinum chemotherapy with a third-generation agent such as docetaxel, paclitaxel, gemcitabine, vinorelbine, and irinotecan.[2,3] Although patients with stage II, IIIA, or IIIB NSCLC receive platinum-based chemotherapy as part of combined modality treatment with thoracic radiotherapy or surgery, many will be candidates for second or third-line chemotherapy. Docetaxel is the only cytotoxic agent with a proven survival advantage over supportive care in patients with disease progression after cisplatin-based chemotherapy for NSCLC.[4] The other agent for which a survival benefit has been demonstrated in this setting is erlotinib,[5] which was approved in Japan for the treatment of relapsed NSCLC in October 2007. Erlotinib is a selective, orally active epidermal growth factor receptor tyrosine kinase inhibitor (EGFR-TKI). In contrast to the experience with the cytotoxic chemotherapeutic agents, response to treatment with EGFR-TKIs has been reported to be influenced by gender, histological type, race or ethnic origin, and smoking status.[5–8]

Tumor molecular markers, including *EGFR* gene mutations and protein expression, have been widely studied in patients with NSCLC, and there is strong evidence that the presence of *EGFR* gene mutations is a predictor of tumor response and resistance.[9–12] However, few prospective studies have evaluated molecular markers as predictors of outcome, and their clinical usefulness is unproven.

This report presents the results of the first phase II study of erlotinib conducted in Japanese patients with NSCLC. The purpose was to evaluate the efficacy and safety of erlotinib in this population. Where available, tumor biopsy samples were analyzed for EFGR-related markers.

PATIENTS AND METHODS

This phase II, multicenter, open-label study recruited patients at 11 hospitals in Japan. The primary end point was the objective response rate (ORR) to erlotinib treatment (150 mg/d). Secondary endpoints were disease control rate (DCR), response duration, time to progression, overall survival (OS), quality of life (QoL), and safety. The protocol was approved by the ethics review boards of all participating institutions, and conducted in accordance with Japanese Good Clinical Practice guidelines.

Patient Selection

Patients with histologically or cytologically documented stage IIIB or IV NSCLC at study entry (not curable with surgery or radiotherapy) that was recurrent or refractory to treatment with one or more chemotherapy regimens (including at least one platinum-containing regimen), were enrolled into this study. Additional eligibility criteria included: the presence of measurable lesions by Response Evaluation Criteria in Solid Tumors (RECIST); age ≥20, <75 years; Eastern Cooperative Oncology Group performance status (ECOG PS) of 0–2, and adequate bone marrow, hepatic, and renal function, i.e., aspartate aminotransferase and alanine aminotransferase (ALT) levels ≤2.5 times the upper limit of normal and total bilirubin of ≤1.5 times the upper limit of normal. Patients with existing or previous interstitial lung disease (ILD) were excluded, although a history of radiation pneumonitis (limited to the field of radiation treatment) was permitted. Concomitant anticancer treatment and prophylactic medication for adverse events (AEs) were not permitted, nor was prior use of anti-EGFR or anti human epidermal growth factor receptor (HER2) agents (small molecules and monoclonal antibodies). Written informed consent was obtained from all patients.

Treatment Procedure

After completion of the baseline assessments (see below), all patients received erlotinib (150 mg orally) each morning, 1 hour before breakfast, until the occurrence of progressive disease (PD) or unacceptable toxicity (all AEs were graded using the National Cancer Institute Common Toxicity Criteria Version 2.0). In the event of treatment-related toxicity, 2 dose reductions of 50 mg were permitted per patient, and dosing could also be interrupted for up to 14 days. For grade 3 or intolerable grade 2 rash, treatment was withheld until the rash improved to grade 2 or less, when a lower dose of erlotinib was initiated. For grade 3 diarrhea, treatment was withheld until the diarrhea was grade 1 or less, when a lower dose was started. For ILD of any grade, or any grade 4 toxicity, treatment was immediately and permanently discontinued.

Evaluation of Efficacy

Objective tumor response was assessed in accordance with RECIST.[13] Tumor assessments were performed at baseline, then every 4 weeks until week 16, and then every 8 weeks thereafter. Confirmation of complete or partial responses (PR) was required, by means of a second assessment conducted 28 days or more after the initial assessment. Stable disease (SD) was defined as disease control (absence of progression) maintained for at least 6 weeks. An independent response evaluation committee consisting of 2 oncologists and a radiologist reviewed images of patients with complete response, PR, and SD. Individual survival times were determined from the survival status of each patient during the study period and at the post study follow-up survey conducted in June-July 2005 and May-July 2006. OS was defined as the time from first administrated to death.

Quality of Life Evaluation

The Functional Assessment of Cancer Therapy-Lung (FACT-L) questionnaire (Version 4-A)[14] was used to assess QoL. The full FACT-L questionnaire was administered at baseline and then every 28 days. In addition, the Lung Cancer Subscale (LCS), an independently validated component of FACT-L, was administered weekly during the treatment period. Best responses on the LCS were analyzed for all patients with a baseline LCS score of 24 or less (out of a possible 28 points) and symptomatic improvement was defined as an increase from the baseline score of 2 or more points, sustained for at least 4 weeks.

Evaluation of Safety

Baseline assessment included a full patient history, physical examination, standard laboratory tests, electrocardiography, chest radiography, pregnancy test, and ophthalmologic tests (vision test and slit-lamp examination). Every week until week 8 and every 2 weeks thereafter, vital signs and ECOG PS were monitored and blood samples were taken for hematology and blood chemistry tests. A radiograph examination to assess pulmonary toxicity was conducted weekly until week 4 and every 2 weeks thereafter. Ophthalmologic examinations were repeated at week 8 and at the end of the study. Observation and evaluation of AEs was conducted as appropriate throughout the study period. All AEs were graded using National Cancer Institute Common Toxicity Criteria Version 2.0. For all ILD-like events, the data safety monitoring board (which consisted of oncologists and pneumonologists) reviewed the clinical data and images; the images were also examined by a review committee of radiologists with expertise in drug-induced pulmonary disorders.

Biomarker Analysis

EGFR mutations and EGFR and HER2 protein expression were assessed in patients with suitable tumor tissue specimens at first diagnosis or surgery; these assessments were done only with separate written consent. Tumor samples were obtained from each center as formalin-fixed and paraffin-embedded blocks, or as thinly sliced tissue sections mounted on glass microscope slides. For the mutation analysis, the tissue was microdissected by Targos Molecular Pathology (Kassel, Germany) and direct sequencing was conducted at the Roche Centre of Medical Genomics (Basel, Switzerland), using a nested polymerase chain reaction of exon 18–21. EGFR protein expression was analyzed by Lab Corp (Mechelen, Belgium). EGFR expression analysis was conducted by immunohistochemistry using Dako EGFR PharmDx™ kits (Dako, Carpinteria, CA). A positive test was

defined as membranous staining in ≥10% of the tumor cells. HER2 protein expression was measured using HercepTest™ (Dako, Carpinteria, CA), and a score of 1+ or above (possible scores were: 0, 1+, 2+, 3+) was regarded as positive.

Statistical Analysis

Given an expected ORR of 20%, a Fisher's exact test was performed (one-sided $\alpha = 2.5\%$). Based on 50 patients, the power to test the null hypothesis (ORR = 5%) was 89.66%. The target sample size of 60 patients was chosen on the expectation that a proportion of patients would prove to be ineligible for the study. The main analysis of efficacy was conducted on the full analysis set (FAS), which was produced by omitting ineligible patients. The 95% confidence interval (CI) for ORR, DCR, and symptom improvement rate was calculated by the Clopper-Pearson method. The time-to-event variables were estimated by the Kaplan-Meier method. Logistic regression and Cox proportional hazards regression analysis was conducted on best response and survival time, respectively. In both cases, univariate and multivariate analyses were used to evaluate the effects of 11 factors relating to patient and disease characteristics, and previous treatment.

RESULTS

Patient Characteristics

A total of 62 patients were enrolled between December 2003 and January 2005. All were evaluable for safety and 60 were evaluable for efficacy (FAS). Two patients did not have a measurable lesion according to RECIST. The baseline characteristics of the patients, including their treatment history, are shown in Table 1. The median age was 60.5 years (range: 28–74 years), and 71% of patients were male. Fifty-seven patients (92%) had adenocarcinoma, and 20 (32%) were never-smokers. Twenty-seven patients (44%) had received only one previous chemotherapy regimen.

Efficacy

Tumor response rates in the FAS (as assessed by extrainstitutional review) are shown in Table 2. Seventeen patients were assessed as having a PR and 13 as having SD. The ORR was 28.3% (95% CI: 17.5–41.4%) and the DCR was 50% (95% CI: 36.8–63.2%). In three patients, objective response could not be adequately confirmed, because each discontinued treatment early in the study due to AEs. The median duration of response was 278 days (95% CI: 203–422 days), and time to progression was 77 days (95% CI: 55–166 days). OS was determined based on information collected until the follow-up survey conducted in May–July 2006. The median survival time was 14.72 months (95% CI: 11.07–20.57 months; 19 censored cases) and the 1-year survival rate was 56.5% (95% CI: 43.9–69.1%) (Figure 1). The median OS of patients with PD was 9.95 months. The symptom improvement rate measured using the LCS was 42.1% (24/57; 95% CI: 29.1–55.9%).

The overall response rate was higher in women (58.8%; 10/17) than in men (16.3%; 7/43, χ^2 test: $p = 0.0029$), and in never-smokers (63.2%; 12/19) than in current or former smokers (12.2%; 5/41, $p = 0.0002$). There was no statistically significant difference between the response rate in patients with adenocarcinoma (28.6%; 16/56) and nonadenocarcinoma histology (25.0%; 1/4, $p = 1.0000$). The response

TABLE 1. Summary of Baseline Patient Characteristics and Demographics

Patient and Disease characteristics	No. of Patients ($n = 62$)	%
Age (yr)		
Median	60.5	
Range	28–74	
Sex		
Female	18	29
Male	44	71
Performance status		
0	20	32
1	41	66
2	1	2
Histology		
Adenocarcinoma	57	92
Squamous cell	4	6
Unclassified	1	2
Stage		
IIIB	8	13
IV	54	87
Smoking history		
Never smoked	20	32
Current- or former smoker	42	68
Time since initial diagnosis (d)		
Median	304.0	
Range	2–2353	
Prior chemotherapy regimens		
1	27	44
2	23	37
≥3	12	19
Prior taxanes		
No	10	16
Yes	52	84
Time since last regimen (d)		
Median	80.0	
Range	29–528	

TABLE 2. Response Assessment

Parameter	n	(%)
Partial response	17	28.3
Stable disease	13	21.7
Progressive disease	27	45.0
Not assessable	3	5.0
Response rate (%) (95% CI)	28.3 (17.5–41.4)	
Disease control rate (%) (95% CI)	50.0 (36.8–63.2)	
Duration of response (median: days)[a] (95% CI)	278 (203.0–422.0)	
Time to progression (median: days)[a] (95% CI)	77 (55–166)	

[a] Kaplan–Meier method.
CI, confidence intervals.

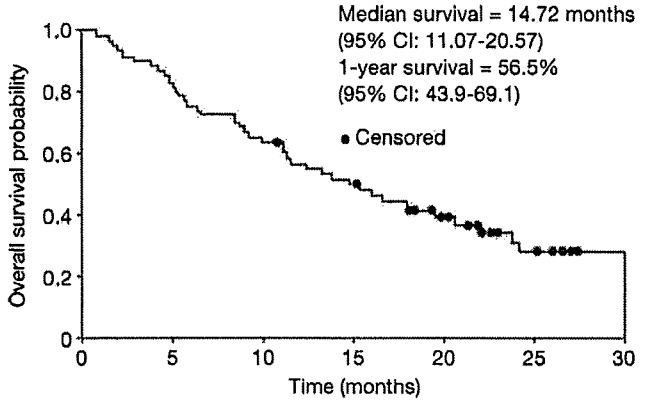

FIGURE 1. Kaplan-Meier plot showing overall survival.

rate was not affected by the number of previous chemotherapy regimens, however, being 27% for patients with one previous regimen (7/26) and 29% for those with 2 or more regimens (10/34). No statistically significant differences were found between other patient subgroups. In a multivariate logistic regression analysis, only smoking history was found to be a statistically significant predictor of response. A multivariate Cox regression analysis showed that both smoking history and ECOG PS were significant predictors for OS (Table 3).

Safety

All 62 patients who received erlotinib were assessed for safety. Treatment-related AEs were observed in all patients, and there were 24 serious AEs in 18 patients (29%). AEs led to discontinuation of erlotinib in 11 patients (18%), including 3 due to ILD-like events, 2 due to ALT elevation, and one each due to rash, paronychia, punctate keratitis, dyspnea/hypoxia, pneumonia and fever/inflammatory neck swelling, and to dose interruptions in 30 patients (48.4%). While the main reasons for the dose interruptions were rash (n = 15; 24.2%) and diarrhea (n = 4; 6.5%), only one patient with rash

TABLE 3. Logistic and Cox Regression Analysis

	Odds Ratio[b]	(95% CI)	p
Logistic regression analysis of response			
Univariate analysis			
Sex (female vs male)	0.14	0.04–0.48	0.002
Age (<65 vs ≥65)	1.26	0.38–4.13	0.704
Histology (non-AD vs AD)	1.20	0.12–12.41	0.878
Smoking history (never vs current or former)	0.08	0.02–0.30	<0.001
Performance status (0 vs ≥1)	0.62	0.19–1.98	0.420
Prior regimens (1 vs ≥2)	1.13	0.36–3.53	0.832
Stage (IIIB vs IV)	0.99	0.17–5.65	0.988
KL-6 (baseline) (<median [496.5 U/ml[a]] vs ≥median)	1.64	0.53–5.12	0.392
Best response to previous chemotherapy (non-PR vs PR)	0.90	0.24–3.33	0.869
Prior taxanes (no vs yes)	0.43	0.10–1.84	0.253
Time since initial diagnosis (≤12 mo vs >12 mo)	1.02	0.31–3.30	0.976
Multivariate analysis			
Smoking history (never vs current or former)	0.06	0.02–0.28	<0.001
Time since initial diagnosis (<12 mo vs ≥12 mo)	2.22	0.49–10.20	0.304
Cox regression analysis of survival			
Univariate analysis			
Sex (female vs male)	1.76	0.85–3.61	0.126
Age (<65 vs ≥65)	0.86	0.44–1.71	0.675
Histology (non-AD vs AD)	0.55	0.19–1.55	0.255
Smoking history (never vs current or former)	1.90	0.93–3.90	0.079
Performance status (0 vs ≥1)	2.31	1.12–4.73	0.023
Prior regimens (1 vs ≥2)	0.93	0.50–1.75	0.833
Stage (IIIB vs IV)	1.38	0.49–3.89	0.542
KL-6 (baseline) (<median [496.5 U/ml[a]] vs ≥median)	1.64	0.87–3.06	0.125
Best response to previous chemotherapy (non-PR vs PR)	0.66	0.31–1.44	0.300
Prior taxanes (no vs yes)	2.09	0.74–5.90	0.163
Time since initial diagnosis (≤12 mo vs >12 mo)	0.76	0.40–1.47	0.418
Multivariate analysis			
Smoking history (never vs current or former)	2.20	1.06–4.56	0.035
Performance status (0 vs ≥1)	2.59	1.25–5.37	0.011

[a] Or 629 ng/ml.
[b] Left site of 'vs' indicates reference group.
PR, partial response; AD, adenocarcinoma; CI, confidence interval.

TABLE 4. Major Treatment-Related Adverse Events and Interstitial Lung Disease-Like Events

Event[a]	n	%	NCI-CTC Grade (n) 1	2	3	>4
Rash	61	98.4	18	41	2	0
Dry skin	50	80.6	44	6	—	—
Diarrhea	46	74.2	33	10	3	0
Pruritus	45	72.6	38	7	0	—
Stomatitis	24	38.7	19	4	1	0
Fatigue	21	33.9	15	6	0	0
Anorexia	19	30.6	11	6	2	0
Paronychia	18	29.0	12	5	1	0
C-reactive protein increased	15	24.2	8	7	0	0
Alanine aminotransferase increased	15	24.2	11	2	2	0
Total bilirubin increased	15	24.2	8	7	0	0
Weight loss	13	21.0	13	0	0	—
ILD-like events	4	6.5	1	0	2	1[b]

Case	Sex	Age	Smoking History	Brinkman Index	Performance Status	Histology	Onset (day)	Outcome	Relation to Erlotinib[c]
1	Male	75	Former	640	1	Adenocarcinoma	52	Recovery	Probable
2	Male	67	Never	—	1	Adenocarcinoma	103	Death (145)	Possible
3	Female	39	Never	—	0	Adenocarcinoma	85	Recovery	Probable
4	Male	69	Former	1000	1	Adenocarcinoma	13	Recovery	Unlikely

[a] Categorized by MedDra Ver.7.1 (except for event).
[b] Grade 5.
[c] Judged by ILD review committee.
NCI-CTC, National Cancer Institute Common Toxicity Criteria; ILD, interstitial lung disease.

had to discontinue treatment, and no patients had to discontinue because of diarrhea or any other digestive toxicity. Fourteen patients (23%) had dose reductions due to AEs, mostly due to rash ($n = 9$; 15%). Treatment-related AEs with an incidence of 20% or more are shown in Table 4; the main events were rash (98%), dry skin (81%), and diarrhea (74%). Elevated laboratory test values related to liver function were found in some patients (total bilirubin: 24%, ALT: 24%), and grade 3 ALT elevation led to treatment discontinuation in 2 patients. Four patients had ILD-like events, including worsening of radiation pneumonitis in one patient, and one died (Table 4). All four (three men; one woman) had an ECOG PS of 0–1 and 2 were former smokers. The patient who died was a 67-year-old man with adenocarcinoma and no history of smoking who discontinued treatment on day 84 due to PD. He developed interstitial pneumonia on day 103 and received 3 days of palliative thoracic irradiation from day 99, after completing the study (3 Gy × 3 days). A computed tomography scan showed characteristic features of ILD (cryptogenic organizing pneumonia-like pattern), and the ILD review committee decided that use of erlotinib could not be excluded as the cause. For the patient with worsening of radiation pneumonitis (case 4), the committee concluded that there was a possible influence of previous radiation therapy, and that this could be seen in the computed tomography scan on day 1. There was, therefore, little reason to suspect that the use of erlotinib had been the cause. Rather, it appeared that the radiation pneumonitis had worsened according to the normal course of illness.

Biomarker Analysis

Tissue samples for measurement of *EGFR* mutations were available for 16 of the 60 patients evaluated for efficacy. For 7 patients, all base sequences were successfully identified in the 4 segments of exons 18–21. All seven (three men, four women) had adenocarcinoma; three were never-smokers, three former smokers and one a current smoker. Three had PR, two SD and two PD. Five of the seven patients had *EGFR* gene mutations and, in all, seven different mutations were detected. The 3 patients with PR all had deletion mutations in exon 19 (del E746-A750 or del S752-I759). One of the 2 patients with PD had no mutations and the other had 2 substitution mutations: L858R in exon 21 and the resistance mutation T790M in exon 20 (Table 5).

Paraffin-embedded tissue samples for immunohistochemistry were available from 12 patients, among whom, 11 had successful determinations of immunohistochemical staining (including 3 patients with PR). Six of the 11 were found to be EGFR-positive and 4 were HER2-positive. However, there were no notable relationships between the EGFR and HER2 expression status and either tumor response or patient characteristics such as sex, histological type or smoking history (data not shown).

DISCUSSION

The present study was conducted on the basis of results from a phase I study of erlotinib in Japanese patients with solid tumors,[15] which showed erlotinib to be well tolerated at

TABLE 5. *EGFR* Mutation Analysis

Response	TTP (d)	Survival (d)	Sex	Histology	Smoking history	Mutation status	Exon	Type of Mutation
PR	222	546	Female	Adenocarcinoma	Never	+	19	del E746–A750
PR	230	811+	Male	Adenocarcinoma	Current	+	19	del S752 –I759 and T751N
PR	278+	911	Female	Adenocarcinoma	Never	+	19	V786M, del E746–A750
SD	224	649+	Male	Adenocarcinoma	Former	+	21	del V834-
SD	77	737	Female	Adenocarcinoma	Former	–	—	—
PD	60	604+	Female	Adenocarcinoma	Never	+	20, 21	L858R, T790M
PD	19	347	Male	Adenocarcinoma	Former	–	—	—

TTP, time to progression; PR, partial response; SD, stable disease; PD, progressive disease.

a dose of 150 mg/d, as well as a phase II study of erlotinib in NSCLC conducted in the United States.[16] In this study, erlotinib achieved an ORR of 28.3%, which was higher than expected, and a DCR of 50%. The response rate was higher than that determined in the above-mentioned phase II study[16] and in keeping with the rate seen in the Japanese subgroup in the phase II study of gefitinib (IDEAL1; 27.5%).[6] Assessment of QoL using the LCS demonstrated a clinically meaningful rate of symptom improvement of 42.1%.

The characteristics of the patients in this study were generally similar to those of NSCLC patients as a whole, in terms of their demographics and disease and treatment history, with the exception of a particularly high proportion of patients with adenocarcinoma (92%). The possibility of enrollment bias on the basis of histological type cannot be ruled out, in part because enrollment coincided with the emergence of reports that the efficacy of EGFR-TKI therapy was greater in patients with adenocarcinoma.[17] However, we also observed one PR and two SDs among three patients with squamous cell carcinoma (FAS population), and our results do not rule out the efficacy of erlotinib in any patient subtype. A multivariate logistic regression analysis showed that smoking status was significantly associated with tumor response, in agreement with previous studies of predictive factors for response to EGFR-TKIs.[5,18,19]

The median survival time with erlotinib was an encouraging 14.7 months. One of the reasons for this long survival may be the high proportion of never-smokers and patients with adenocarcinoma compared with those of other studies, particularly the multinational phase III erlotinib study (BR.21).[5] On the other hand, the presence of *EGFR* gene mutations is currently regarded as an important determinant of treatment response to EGFR-TKIs[20,21] and may be the most important factor in relation to the favorable results seen in the present study. However, it is important to recognize that the potential prognostic effect of mutation status cannot be excluded. The sample size of this and previous trials limits the interpretation of this effect, which will be adequately assessed only by means of appropriately powered trials specifically designed to examine these factors.

Assessment of the presence or absence of *EGFR* gene mutation was possible in only seven patients in the present study. Despite this, the results were consistent with the results of some previous studies. All three of the patients who had a PR (including a male current smoker) had an in-frame deletion in exon 19, which is considered to be the most frequent mutation site in the EGFR-TK domain.[22] One of the 2 patients with PD had a point substitution mutation (L858R) in exon 21, the second most frequent mutation site,[22] and a point mutation (T790M) in exon 20, which is suggested to be involved in tolerance to EGFR-TKI.[12,23,24] It would be valuable to conduct further prospective randomized studies on the association between these markers and survival during treatment with erlotinib in Japanese patients.

Rash and diarrhea were the main AEs reported by patients on erlotinib treatment, as reported in previous studies.[5,15,16] Rash was observed in almost all patients, and was the main reason for treatment interruptions or dose reductions. Although the protocol allowed treatment to be interrupted for grade 3 rash (or intolerable grade 2 rash), grade 3 rash only occurred in 2 patients, leading to discontinuation of treatment in one. Most cases of rash responded to symptomatic treatment and either interruption or dose reduction of erlotinib. Despite suggestions in some reports that the presence of erlotinib-related rash is associated with treatment efficacy and can be used to predict response,[25] no supportive evidence was found in the present study.

The incidence of ILD, which is the most clinically problematic AE associated with erlotinib, tended to be higher than that reported in other clinical studies of erlotinib.[5,26] This is in keeping with this class of agent, and is not unexpected in the Japanese population.

We would recommend that careful screening of patients for ILD risk factors, particularly signs of interstitial pneumonia and pulmonary fibrosis, is done before erlotinib therapy is initiated. Individuals with any previous history of ILD were excluded from this study.

In conclusion, erlotinib (150 mg/d) was shown to have promising antitumor efficacy in Japanese patients with previously treated NSCLC, leading to clinically meaningful improvements in symptoms and an encouraging median survival time. Despite, as expected, a high rate of rash and diarrhea, erlotinib was well tolerated at a dose of 150 mg/d by the majority of patients.

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

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Treatment

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.

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 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.

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

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 mutations 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.

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

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

Table 4 Summary of thymoma and thymic carcinoma patients with *EGFR* or *KIT* mutations in their tumors

Clinical characteristics						Mutation				IHC
No.	Age/sex	Smoking status	Masaoka stage	Histology	Clinical outcome	Gene	Exon	Nucleotide change	Amino acid change	
1	65/F	Never	II	Thymoma (type A)	3 years of disease-free survival after complete resection	*EGFR*	21	2573T > G	L858R	EGFR (+)
2	69/F	Never	III	Thymoma (type B1)	5 years of disease-free survival after complete resection	*EGFR*	21	2588G > A	G863D	EGFR (−)
3	59/M	Former (20 pack-years)	I	Thymic carcinoma (Sq)	6 years of disease-free survival after complete resection	*KIT*	11	1748T > C	L576P	KIT (+)

Abbreviations: Sq, squamous cell carcinoma; IHC, immunohistochemistry.

Fig. 1 Electropherograms of the products of direct sequencing of *EGFR* and *KIT*. (a and b) Two thymomas contained a single missense point mutation in exon 21 of *EGFR*. (c) One thymic carcinoma contained a single missense point mutation in exon 11 of *KIT*.

carcinomas because of the low frequency of *EGFR* or *KIT* mutations in these tumors.

Remarkably, the *EGFR* mutations (L858R and G863D, respectively, in exon 21) observed in the 2 thymomas in our study were similar to the active mutations in NSCLC that have been reported to be predictors of a therapeutic response to EGFR-TKI by NSCLCs [9,21]. Moreover, the *KIT* mutation (L576P in exon 11) identified in the 1 thymic carcinoma in our study had previously been described as one of the mutations that predicts a clinical response of GISTs to

imatinib [22]. We therefore speculate that patients whose thymoma or thymic carcinoma harbors *EGFR* or *KIT* mutations may profit from molecularly targeted therapy with a TKI of EGFR or KIT.

In conclusion, our findings indicate that somatic mutations of *EGFR* or *KIT* of the thymomas and thymic carcinomas are presented in a small number of patients. Further investigation is warranted to determine the susceptibility of such tumors to TKI therapy.

Conflict of interest

None declared.

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Minireview

Emerging ethnic differences in lung cancer therapy

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Although global clinical trials for lung cancer can enable the development of new agents efficiently, whether the results of clinical trials performed in one population can be fully extrapolated to another population remains questionable. A comparison of phase III trials for the same drug combinations against lung cancer in different countries shows a great diversity in haematological toxicity. One possible reason for this diversity may be that different ethnic populations may have different physiological capacities for white blood cell production and maturation. In addition, polymorphisms in the promoter and coding regions of drug-metabolising enzymes (e.g., CYP3A4 and UGT1A1) or in transporters (e.g., ABCB1) may vary among different ethnic populations. For example, epidermal growth factor receptor (EGFR) inhibitors are more effective in Asian patients than in patients of other ethnicities, a characteristic that parallels the incidence of EGFR-activating mutations. Interstitial lung disease associated with the administration of gefitinib is also more common among Japanese patients than among patients of other ethnicities. Although research into these differences has just begun, these studies suggest that possible pharmacogenomic and tumour genetic differences associated with individual responses to anticancer agents should be carefully considered when conducting global clinical trials.
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Lung cancer is the most common malignancy worldwide. Approximately 1.2 million people are diagnosed with lung cancer annually (accounting for 12.3% of all cancers); the second most common malignancy is breast cancer (10.4%), followed by colorectal cancer (9.4%). As lung cancer almost invariably has a poor prognosis, it is the largest single cause of death from cancer in the world, with a mortality of 1.1 million annually (Stewart and Kleihues, 2003). Only 15% of lung cancer patients have a disease that is confined to the lung and are candidates for surgical resection; most patients with this disease have distant metastases or pleural effusion at the time of their initial diagnosis. These patients can be treated with systemic chemotherapy, but the efficacy of currently available anticancer agents is limited and patients with advanced diseases rarely live long.

As the development of new anticancer agents and chemotherapeutic regimens is both time and money consuming, clinical trials need to be as efficient as possible. One effort in this direction has been the adoption of global clinical trials for new agents that involve trial centres on more than one continent; this strategy enables adequate sample sizes to be obtained in a relatively short-time period and eliminates the need for redundant clinical trials with similar objectives conducted in different countries. However, whether the results of clinical trials performed in one population can be fully extrapolated to other populations remains questionable because of potential differences in trial designs, study-specific criteria, patient demographics, frequency of monitoring, and population-related pharmacokinetics, pharmacodynamics and pharmacogenomics. Recently, these genetic and physiologic factors influencing cancer chemotherapy have been increasingly examined and reported.

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CLINICAL OBSERVATIONS OF TOXICITY DURING CYTOTOXIC CHEMOTHERAPY

A comparison of phase III trials for the same drug combinations against non-small cell lung cancer conducted in different countries shows a great diversity in toxicity (Sekine *et al*, 2006). Among trials studying the combination of carboplatin and paclitaxel, the dose of carboplatin was fixed in all the trials, but the dose of paclitaxel was 200 mg m^{-2} in Japanese and European trials and 225 mg m^{-2} in American trials. Grades 3–4 neutropenia was noted in 88% of the patients in the Japanese trial, 15–51% of the patients in the European trials, and 6–65% of the patients in the American trials. Meanwhile, grades 3–4 febrile neutropenia was encountered in 16% of the patients in the Japanese trial, 0–9% of the patients in the European trials, and 2–4% of the patients in the American trials (Table 1). For combinations of cisplatin and docetaxel (Table 1) and cisplatin and vinorelbine (Table 2), the incidences of grades 3–4 neutropenia and febrile neutropenia were almost the same between phase III trials performed in different areas, but the doses of docetaxel and vinorelbine in the Japanese trials were lower than those in the European and American trials. Thus, neutropenia in patients receiving a combination of platinum and antimicrotubule agents may be more severe in Japanese than in Europeans and Americans. A higher frequency of grades 3–4 neutropenia in Japanese patients than in American patients was associated with combinations of cisplatin and irinotecan (65 *vs*

Table 1 Toxicity associated with a combination of platinum and taxane

Research group	Chemotherapy dose		No. of patients	Grades 3–4 toxicity (%)		Reference
	Platinum	Taxane		NP	FNP	
A combination of carboplatin and paclitaxel						
Japan	6 (AUC)	200 ($mg\,m^{-2}$)	145	88	16	Ohe *et al* (2007)
Greece	6 (AUC)	200 ($mg\,m^{-2}$)	252	15	0	Kosmidis *et al* (2002)
EU	6 (AUC)	200 ($mg\,m^{-2}$)	309	51	4	Rosell *et al* (2002)
ECOG	6 (AUC)	225 ($mg\,m^{-2}$)	290	63	4	Schiller *et al* (2002)
SWOG	6 (AUC)	225 ($mg\,m^{-2}$)	206	57	2	Kelly *et al* (2001)
SWOG	6 (AUC)	225 ($mg\,m^{-2}$)	182	—	3	Gandara *et al* (2004)
USA	6 (AUC)	225 ($mg\,m^{-2}$)	190	65	—	Belani *et al* (2005)
USA	6 (AUC)	225 ($mg\,m^{-2}$)	345	6	—	Herbst *et al* (2004)
A combination of cisplatin and docetaxel						
Japan	80 ($mg\,m^{-2}$)	60 ($mg\,m^{-2}$)	151	74	2	Ohe *et al* (2007)
ECOG	75 ($mg\,m^{-2}$)	75 ($mg\,m^{-2}$)	289	69	11	Schiller *et al* (2002)
USA	75 ($mg\,m^{-2}$)	75 ($mg\,m^{-2}$)	408	75	5	Fossella *et al* (2003)

NP, neutropenia; FNP, febrile neutropenia.

Table 2 Toxicity associated with a combination of cisplatin and vinorelbine

Research group	Chemotherapy dose ($mg\,m^{-2}$)		No. of patients	Grades 3–4 toxicity (%)		Reference
	Cisplatin	Vinorelbine		NP	FNP	
Japan	80 (day 1)	25 (days 1, 8)	145	88	18	Ohe *et al* (2007)
Greece	80 (day 8)	30 (days 1, 8)	204	37	11	Georgoulias *et al* (2005)
France	100 (day 1)	30 (weekly)	156	83	22	Pujol *et al* (2005)
EU	120 (day 1)	30 (weekly)	206	79	4	Le Chevalier *et al* (1994)
SWOG	100 (day 1)	25 (weekly)	202	76	1	Kelly *et al* (2001)
USA	100 (day 1)	25 (weekly)	404	79	5	Fossella *et al* (2003)

NP, neutropenia; FNP, febrile neutropenia.

32%, $P<0.001$) and cisplatin and etoposide (92 *vs* 66%, $P<0.001$) for the treatment of extensive small-cell lung cancer (Lara *et al*, 2007).

How can this ethnic difference in the severity of neutropenia be explained? One possibility is that the physiological capacity of the white blood cell production and maturation may vary among different ethnic populations. An asymptomatic reduction in neutrophils (benign neutropenia) is more commonly observed in individuals of African descent than in Caucasians, and no data on this phenomenon are available for Asians (Hsieh *et al*, 2007). The mechanisms are unclear, but a lower bone marrow reserve, an intrinsic marrow difference, an abnormal cytokine response, or any combination of these factors have been suggested (Hsieh *et al*, 2007). The lower neutrophil counts were associated with higher levels of IL-8 and granulocyte colony-stimulating factor in African volunteers. Thus, these cytokines are considered to compensate for the relatively low neutrophil counts in this population (Mayr *et al*, 2007). A recent report showed that ethnicity-related low neutrophil counts were associated with neutrophil elastase (ELA2) polymorphisms (C-199A), but not with serum cytokine levels (Grann *et al*, 2007).

ETHNIC DIFFERENCES IN DRUG METABOLISING ENZYMES

An explanation for the ethnic differences in haematological toxicity may be the varying activities of drug-metabolising enzymes and transporters that are mainly associated with polymorphisms in the promoter and coding regions of these enzymes (Fujita and Sasaki, 2007). The haematological toxicity of docetaxel monotherapy was associated with the clearance of this agent in Asian patients, a phenomenon that can be largely explained by CYP3A4 activity (Yamamoto *et al*, 2000). A study conducted in the Netherlands showed that docetaxel clearance was associated with the homozygous C1236T polymorphism in the ABCB1 (p-glycoprotein) gene (ABCB1*8) but was not associated with any CYP3A4 gene polymorphisms (Bosch *et al*, 2006). In contrast, docetaxel pharmacokinetics were not associated with the percent decrease in neutrophil counts nor with any polymorphisms in the CYP3A4 and ABCB1 genes in American patients (Lewis *et al*, 2007). Another example of ethnic differences in drug-metabolising enzymes is the association between polymorphisms in genes involved in irinotecan metabolism and irinotecan-induced neutropenia. Among the patients who received irinotecan with or without another anticancer agent, grade 4 neutropenia was noted in 40–57% of the patients with UDP-glucuronosyltransferase (UGT) 1A1*28 (a polymorphism in the promoter region of the UGT1A1 gene) homozygosity, whereas neutropenia was only observed in 15% or less of the patients with wild-type alleles. This association was consistent in both Asian and Caucasian patients, although the frequency of homozygosity was about 10% in Caucasians and much lower in Asians. The UGT1A1*6 allele is another polymorphism at exon 1 that is associated with defective glucuronidating function and is found almost exclusively in Asian individuals with a frequency as high as 20% (Fujita and Sasaki, 2007). UGT1A1*6 is significantly linked to polymorphisms of UGT1A7 and UGT1A9. A haplotype including UGT1A1*6 and UGT1A7*3, noted in as many as 15% of Japanese patients, and UGT1A1*6 homozygosity, noted in 7% of Korean patients, were significantly associated with decreased glucuronosyltransferase activity for SN-38 and severe neutropenia (Han *et al*, 2006; Fujita

et al, 2007). In 177 Japanese patients treated with irinotecan-including chemotherapy, a homozygous or double heterozygous genotype for UGT1A1*6 and UGT1A1*28 (*6/*6, *28/*28 or *6/*28) was significantly associated with severe neutropenia (Minami et al, 2007). In addition, patients with a homozygous C3435T polymorphism in the ABCB1 gene are four-fold more likely to develop grade 3 diarrhoea when treated with a combination of cisplatin and irinotecan (Lara et al, 2007).

Data on associations between polymorphisms in genes coding drug-metabolising enzymes and therapeutic efficacy remain scarce. A recent prospective study in 250 patients with metastatic colorectal cancer showed a significantly higher response rate (67 *vs* 40%) and a nonsignificant survival advantage (hazard ratio (HR): 0.81; 95% confidence interval (CI): 0.45–1.44) in patients homozygous for UGT1A1*28, compared with those with wild-type alleles; these outcomes were associated with a higher exposure to SN-38 (Toffoli et al, 2006). In a study of 81 NSCLC patients, those who were homozygous for UGT1A1*6 had a lower response rate (0 *vs* 50%, $P=0.038$) and a poorer MST (7.6 *vs* 17.7 months, $P=0.017$) as well as greater toxicities than the other patients (Han et al, 2006). The most plausible explanation for the negative effects of UGT1A1*6 on treatment outcome may be that the dose intensity or cycle number might have been reduced in patients with UGT1A1*6 because of polymorphism-associated toxicities (Fujita and Sasaki, 2007).

These pharmacogenetic analyses have been rather preliminary. Data on genotyping, pharmacokinetics, and pharmacodynamics collected from a large number of patients with different ethnic backgrounds are needed to demonstrate the cause of ethnic differences in chemotherapy-associated toxicity.

EFFICACY OF EPIDERMAL GROWTH FACTOR RECEPTOR TYROSINE KINASE INHIBITORS

Epidermal growth factor receptor (EGFR), a cell membrane receptor with tyrosine kinase activity, is expressed in most patients with NSCLC and plays a role in cellular proliferation, inhibition of apoptosis, angiogenesis, metastatic potential, and chemoresistance. Small-molecule inhibitors of EGFR, such as gefitinib and erlotinib, have shown antitumor activity and have alleviated symptoms in NSCLC patients who were previously treated with standard chemotherapy. Two randomized phase II studies, IDEAL (Iressa Dose Evaluation in Advanced Lung Cancer)-1 (involving 210 patients and conducted in Europe, Australia, South Africa, and Japan) and IDEAL-2 (involving 216 patients and conducted in the USA), have evaluated the efficacy of gefitinib at a dose of either 250 mg daily or 500 mg daily in patients with advanced NSCLC in whom earlier platinum-based chemotherapy had failed. No difference in the response rates between the doses was noted, but an increased response rate was recorded for never smokers, women, and those with an adenocarcinoma histology, compared with patients who did not have these characteristics. In addition, the response rate was 28% in Japanese patients but only 9–12% in patients of other ethnicities (Fukuoka et al, 2003; Kris et al, 2003). A randomized phase III trial, ISEL (Iressa Survival Evaluation in Lung Cancer), of gefitinib *vs* a placebo in 1692 NSCLC patients who had been previously treated with one or two chemotherapeutic regimens failed to show any survival benefit of gefitinib; in the overall population, the median survival times (MSTs) in the gefitinib and placebo arms were 5.6 and 5.1 months, respectively (HR: 0.89; 95% CI: 0.78–1.03). A subgroup analysis, however, showed that the MST was longer in Asian patients receiving gefitinib than in those receiving the placebo (MST: 9.5 *vs* 5.5 months; HR: 0.66; 95% CI: 0.48–0.91). Similar results were seen for never smokers: patients receiving gefitinib survived longer than those receiving the placebo (MST: 8.9 *vs* 6.1 months; HR: 0.67, 95% CI: 0.49–0.91) (Thatcher et al, 2005).

A similar association between objective responses and ethnicity was observed in studies on erlotinib monotherapy for previously treated advanced NSCLC. In an American phase II trial of this agent in 57 advanced NSCLC patients with disease progression or relapse after platinum-based chemotherapy, the response rate was 12% and the MST was 8.4 months (Perez-Soler et al, 2004). In contrast, the combined data of two Japanese phase II trials of erlotinib in similar patient populations showed objective responses in 30 of 106 (28%) patients and an MST of 13.8 months. Among the responders, significantly higher proportions of females (50%) than males (17%) ($P=0.0009$) and of never smokers (51%) than smokers (14%) were observed ($P<0.0001$) (Tamura et al, 2007). A phase III trial of erlotinib or a placebo in 731 NSCLC patients previously treated with one or two chemotherapy regimens showed that the response rate in Asian patients was higher than that in patients of other ethnicities (28 *vs* 10%, $P=0.02$) (Shepherd et al, 2005).

These results of phases II and III trials consistently suggest that EGFR tyrosine kinase inhibitors may be more effective in Asian patients than in patients of other ethnicities.

In April 2004, the activating mutations of the EGFR gene were identified in NSCLC specimens, and cancers with these mutations were reported to be highly sensitive to gefitinib. The populations with higher responses to gefitinib (females, non-smokers and patients with an adenocarcinoma histology) also have higher incidences of EGFR mutations (Kosaka et al, 2004; Pao et al, 2004; Shigematsu et al, 2005). The incidence of EGFR mutations in surgically resected tissue samples is summarised in Table 3 (Kosaka et al, 2004; Pao et al, 2004; Marchetti et al, 2005; Qin et al, 2005; Shigematsu et al, 2005; Soung et al, 2005; Tokumo et al, 2005; Yang et al, 2005; Sasaki et al, 2006). The incidence varies from one report to another, but EGFR mutations tend to be more common among patients with an adenocarcinoma histology and among non-smokers. Among Asian patients, the average incidences of EGFR mutations were 31% overall, 47% among patients with adenocarcinoma, and 56% among non-smokers; among other ethnic populations, however, the average incidences were 7–8% overall, 13–15% among patients with adenocarcinoma, and 34–35% among non-smokers (Table 3). Thus, the percentage of responders to gefitinib or erlotinib almost paralleled the percentage of patients with EGFR mutations.

The mechanism responsible for the high frequency of EGFR mutations in Asian patients is a subject of great interest, and polymorphisms in the regulatory sequence of the EGFR gene have been vigorously investigated. The CA simple sequence repeat 1 (CA-SSR1), a highly polymorphic locus containing 14–21 CA dinucleotide repeats, is located at the 5′ end of intron 1 of the EGFR gene. Studies of CA-SSR1 repeat length and EGFR expression in breast cancer tissues have shown a constant decline in EGFR expression with increasing repeat length (Buerger et al, 2000, 2004). In addition, a shorter repeat length was associated with an elevated risk of lung cancer (Zhang et al, 2007) and poor survival in NSCLC patients (Dubey et al, 2006). The CA-SSR1 repeat length distribution varies according to ethnicity, with Asians tending to have longer repeats than Americans (Liu et al, 2003). Two single-nucleotide polymorphisms in the promoter region of the EGFR gene (−219G/T and −191C/A) were also associated with promoter activity and EGFR expression (Liu et al, 2005), and their polymorphic types (associated with low EGFR expression) were more common among Asians than among other ethnicities (Nomura et al, 2007). These observations suggest that many Asians have polymorphic types that lead to a decreased intrinsic production of EGFR protein. If a certain critical level of EGFR is required to drive the cell toward a malignant phenotype, another mechanism including activating mutations of EGFR and/or the autonomous activation of downstream signalling may be required for the development of lung cancer among Asians (Nomura et al, 2007).

Table 3 Incidence of EGFR mutations in surgically resected specimens

		All cases		Adenocarcinoma		Non-smokers	
Author	Country	Total N	Mutation N (%)	Total N	Mutation N (%)	Total N	Mutation N (%)
Western areas							
Shigematsu	USA	80	11 (14)	44	11 (25)	26	7 (27)
Pao	USA	96	11 (11)	72	11 (15)	15	7 (47)
Yang	USA	219	26 (12)	164	25 (15)	34	12 (35)
Marchetti	Italy	860	39 (5)	375	39 (10)	103[a]	23 (22)
	Subtotal	1255	87 (7)	655	86 (13)	75	26 (35)
Asian areas							
Shigematsu	Japan	263	71 (27)	154	67 (44)	78	47 (60)
Kosaka	Japan	277	111 (40)	224	110 (49)	112[a]	76 (68)
Tokumo	Japan	120	38 (32)	82	37 (45)	36	25 (69)
Sasaki	Japan	95	35 (37)	71	32 (45)	36	25 (69)
Shigematsu	Taiwan	93	32 (34)	55	31 (56)	55	27 (49)
Qin	China	41	10 (24)	17	7 (41)	21	6 (29)
Soung	Korea	153	30 (20)	69	26 (38)	54	25 (46)
Shigematsu	Others	361	107 (30)	214	102 (48)	135	76 (56)
	Subtotal	1403	434 (31)	886	412 (47)	415	231 (56)
Other areas							
Shigematsu	Australia	83	6 (7)	36	5 (14)	7	4 (57)
Shigematsu	Others	158	13 (8)	75	12 (16)	31	9 (29)
	Subtotal	241	19 (8)	111	17 (15)	38	13 (34)
	Toatl	2899	540 (19)	1652	515 (31)	528	270 (51)

[a]Including only patients with adenocarcinoma histology.

INTERSTITIAL LUNG DISEASE ASSOCIATED WITH GEFITINIB AND ERLOTINIB

The frequencies of grades 3–4 common toxicities after the administration of gefitinib, including diarrhoea, skin rash, and elevated liver transaminase levels, have been similar among study populations, but the incidence of severe interstitial lung disease (ILD) associated with the administration of gefitinib differs between patients in Japan and those in other countries. In the IDEAL studies, two Japanese patients developed grades 3–4 ILD (2%), whereas no patients outside of Japan experienced ILD (Fukuoka *et al*, 2003; Kris *et al*, 2003). A retrospective study of 1976 consecutive patients treated with gefitinib at 84 institutions showed that the incidence of ILD was 3.5% and the mortality rate was 1.6%. Several risk factors for the development of gefitinib-induced ILD were identified in the Japanese population: a history of pulmonary fibrosis, a history of smoking, a poor performance status, and a male sex (Ando *et al*, 2006). A similar incidence of ILD (4.6%) was also noted in association with erlotinib chemotherapy in Japanese phase II trials (Tamura *et al*, 2007).

The association between ILD and anticancer treatment is a major topic in Japan because (1) the diagnosis of ILD can be difficult and a consensus among physicians is sometimes not reached, (2) the risk factors for ILD have not been fully established, 3) an effective treatment for ILD has not been established and the condition is often fatal, and (4) the low frequency of this complication makes it difficult to conduct pertinent clinical trials. Gefitinib-induced ILD seems to be more common among Japanese patients than among other patients, but the reasons for this ethnic difference are totally unknown.

CONCLUSION

The findings discussed here suggest that considerable variations in the toxicity and efficacy of anticancer agents may exist among patients of different ethnicities. Although research into these differences has just begun, these studies suggest that possible pharmacogenomic and tumour genetic differences associated with individual responses to anticancer agents should be carefully considered when conducting global clinical trials.

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