Table 4. Reports of interstitial lung disease due to docetaxel plus gemcitabine regimen

Author	Year	Study type	Treatment schedule	n	Grades 3–4 ILD (%)	TRD (%)
Rebattu et al. [13]	2001	Phase I/II	Docetaxel (60, 75, 85, 100 mg/m^2) day 8; gemcitabine (1000 mg/m^2), days 1 and 8, every 3 weeks	49	3 (6.1)	0
Kouroussis et al. [25]	2004	Phase I	Docetaxel (30, 35, 40 mg/m^2), days 1, 8 and 15; gemcitabine (700, 800, 900, 1000 mg/m^2), days 1, 8 and 15, every 4 weeks	26	6 (23)	2 (7.7)
Matsui et al. [21]	2005	Phase I/II	Docetaxel (50, 60 mg/m^2) day 1 or 8; gemcitabine (800, 1000 mg/m^2), days 1 and 8, every 3 weeks	59	3 (5.1)	0
Pujor et al. [27]	2005	Phase III	Docetaxel (85 mg/m^2) day 8; gemcitabine (1000 mg/m^2), days 1 and 8, every 3 weeks	155	8 (5.2)	1 (0.6)
			Cisplatin (100 mg/m^2) day 1; vinorelbine (30 mg/m^2), days 1, 8, 15 and 22, every 4 weeks	156	1 (0.6)	0
Takeda (present study)	2008	Phase III	Docetaxel (60 mg/m^2) day 8; gemcitabine (800 mg/m^2), days 1 and 8, every 3 weeks	65	8 (12.3)	3 (4.6)
			Docetaxel (60 mg/m^2) day 1, every 3 weeks	64	0 (0)	0

ILD, interstitial lung disease; TRD, treatment-related death.

benefit in patients with recurrent advanced NSCLC. The development of less toxic and more effective chemotherapeutic agents, including molecular targeted drugs, is warranted for the second-line treatment of NSCLC.

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appendix

The following institutions participated in the study: Hokkaido Cancer Center (Sapporo), Ibaragi Prefectural Central Hospital (Kasama), Tochigi Cancer Center (Utsunomiya), Nishigunma National Hospital (Shibukawa), Gunma Prefectural Cancer Center Hospital (Ohta), Saitama Cancer Center Hospital (Ina), National Cancer Center Hospital East (Kashiwa), National Cancer Center Hospital (Tokyo), International Medical Center of Japan (Tokyo), Cancer Institute Hospital (Tokyo), Toranomon Hospital (Tokyo), Kanagawa Cancer Center Hospital (Yokohama), Yokohama Municipal Hospital (Yokohama), Niigata Cancer Center Niigata Hospital (Niigata), Gifu Municipal Hospital (Gifu), Aichi Cancer Center Hospital (Nagoya), Nagoya National Hospital (Nagoya), Prefectural Aichi Hospital (Okazaki), Osaka City University Medical School (Osaka), Kinki University School of Medicine (Osaka-Sayama), Osaka Medical Center for Cancer and Cardiovascular Disease (Osaka), Osaka Prefectural Medical Center for Respiratory and Allergic disease (Habikino), Kinki-Chuo Chest Medical Center (Sakai), Toneyama National Hospital (Toyonaka), Osaka Prefectural General Hospital (Osaka), Osaka City General Hospital (Osaka), Kobe City General Hospital (Kobe), Hyogo Collage of Medicine (Nishinomiya), Hyogo Cancer Center (Akashi), Shikoku Cancer Center Hospital (Matsuyama), Kyusyu University Hospital (Fukuoka), and Kumamoto Regional Medical Center (Kumamoto).

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A Randomized, Double-Blind, Phase IIa Dose-Finding Study of Vandetanib (ZD6474) in Japanese Patients With Non-Small Cell Lung Cancer

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Introduction: Vandetanib (ZACTIMA™) is a once-daily, oral anticancer drug that selectively inhibits vascular endothelial growth factor receptor (VEGFR) and epidermal growth factor receptor (EGFR) signaling. Vandetanib was evaluated as a monotherapy in a randomized, double-blind, dose-finding study in Japan.

Patients and Methods: Eligible patients with locally advanced or metastatic (stage IIIB/IV) or recurrent non-small cell lung cancer, previously treated with chemotherapy, were randomized to receive once-daily oral vandetanib 100, 200, or 300 mg (1:1:1). The primary objective was to determine the objective response rate for each vandetanib dose.

Results: Fifty-three patients received vandetanib (100 mg, $n = 17$; 200 mg, $n = 18$; 300 mg, $n = 18$). The objective response rate in each dose arm was 17.6% (3 of 17; 100 mg), 5.6% (1 of 18; 200 mg), and 16.7% (3 of 18; 300 mg). Common adverse events included rash, diarrhea, hypertension, and asymptomatic QTc prolongation. The adverse event profile was generally consistent with that reported previously for agents that inhibit the VEGFR or EGFR signaling pathways. Among the three responders evaluated for *EGFR* mutation, two had no mutation, and in one case, the *EGFR* mutation status could not be determined by direct DNA sequencing and amplification refractory mutation system assay of *EGFR* exons 19–21. Baseline plasma VEGF levels appeared to be lower in patients who experienced clinical benefit after vandetanib treatment.

Conclusion: In Japanese patients with advanced non-small cell lung cancer, vandetanib monotherapy (100–300 mg/d) demonstrated antitumor activity with an acceptable safety and tolerability profile.

Key Words: Non-small cell lung cancer, Vandetanib, EGFR, VEGFR.

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Non-small cell lung cancer (NSCLC) accounts for approximately 75% of lung cancers and is the leading cause of cancer-related death worldwide.[1] Despite the introduction of more effective chemotherapeutic agents, new approaches are required to further improve patient outcome and survival. A major focus of new anticancer research is the targeting of cell-signaling pathways that contribute to tumor growth and progression.

Vascular endothelial growth factor receptor (VEGFR) and epidermal growth factor receptor (EGFR) are key drivers of tumor angiogenesis and cell proliferation, respectively, and both pathways have been validated as clinically relevant targets in NSCLC. The addition of bevacizumab, a humanized anti-VEGF-A monoclonal antibody, to paclitaxel and carboplatin has demonstrated clinical benefit in patients with NSCLC,[2] and the EGFR inhibitors gefitinib and erlotinib have demonstrated clinical activity as single agents in NSCLC.[3,4] Furthermore, EGFR is known to regulate the production of VEGF and other proangiogenic factors[5] and resistance to EGFR inhibition has been associated with increased expression of VEGF in a human tumor xenograft model of NSCLC.[6] Therefore, targeting the VEGFR and EGFR pathways may be more effective than inhibiting either pathway alone. This hypothesis is supported by the promising results from early clinical evaluation of erlotinib and bevacizumab in combination in patients with recurrent NSCLC.[7]

Vandetanib (ZACTIMA™) is a once-daily, orally available anticancer drug that inhibits VEGFR- and EGFR-dependent signaling,[8] as well as the RET (REarranged during

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Disclosure: Haiyi Jiang is an employee of AstraZeneca. All other authors declare no conflict of interest.

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Transfection) receptor tyrosine kinase, which is an important growth driver in certain types of thyroid cancer.[9] Early clinical evaluation of vandetanib has demonstrated a promising efficacy and safety profile in a broad population of patients with advanced cancer. Phase I studies in advanced solid tumors conducted in the USA/Australia[10] and Japan[11] showed that once-daily doses of vandetanib (up to and including 300 mg) were generally well tolerated. In the Japanese study, objective tumor responses were observed in 4 of 9 patients with refractory NSCLC. Subsequent phase II studies in advanced NSCLC demonstrated antitumor activity both as a monotherapy and in combination with certain chemotherapy.[12–14] The positive outcome of these phase II trials led to the ongoing phase III evaluation of vandetanib in previously treated advanced NSCLC.

The primary objective of this randomized phase IIa study was to assess the objective response rate (ORR) to vandetanib (100, 200, or 300 mg/d) in Japanese patients with refractory NSCLC. The three doses investigated were selected based on the outcome of the Japanese phase I trial.[11]

PATIENTS AND METHODS

Patients

Patients with histologic or cytologic confirmation of locally advanced/metastatic (stage IIIB/IV) or recurrent NSCLC after failure of 1 or 2 platinum-based chemotherapy regimens were recruited from eight centers in Japan. The main eligibility criteria were age ≥20 years, a WHO performance status of 0 to 2, an estimated life expectancy ≥12 weeks, and completion of prior chemotherapy and/or radiotherapy at least 4 weeks before study entry (8 weeks for chest radiation and 6 weeks for mitomycin C). Patients with squamous cell histology were also eligible, and brain metastases were permitted if patients were asymptomatic and did not require corticosteroid treatment. Key exclusion criteria were a mixed small-cell and non-small cell histology, evidence of severe or uncontrolled systemic diseases, poorly controlled hypertension, a QTc interval ≥460 milliseconds by electrocardiogram during the screening period, and prior treatment with EGFR or VEGFR signaling inhibitors. All patients provided written informed consent. The study was conducted in accordance with the ethical principles stated in the Declaration of Helsinki, applicable guidelines on good clinical practice, local Institutional Review Board approval, and the AstraZeneca policy on Bioethics.

Study Design and Treatments

This was a randomized, double-blind, parallel-group, phase IIa dose-finding multicenter study to assess the efficacy and safety of vandetanib. A total of 53 patients were randomized (1:1:1) to receive once-daily oral vandetanib (100, 200, or 300 mg/d; Figure 1). Patients were stratified by histology (adenocarcinoma versus others), gender (male versus female), and smoking history (smoker versus nonsmoker). Treatment continued until a withdrawal or dose-interruption criterion was met. These criteria included progressive disease (PD), unacceptable toxicity, protocol noncompliance, or voluntary discontinuation by the patient.

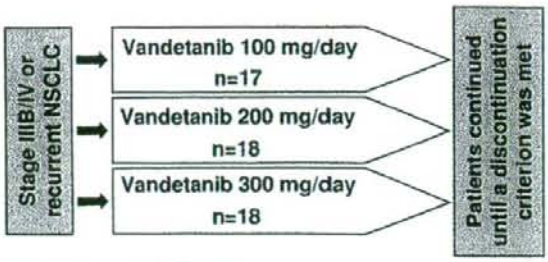

FIGURE 1. Study design.

Efficacy

The primary objective of the study was to determine ORR with vandetanib monotherapy, using the Response Evaluation Criteria in Solid Tumors (RECIST); assessments were performed at baseline and every 4 weeks for the first 24 weeks of treatment, and then every 8 weeks until withdrawal. A confirmed complete response or partial response (PR) was considered to be an objective tumor response. Investigator assessment of best overall tumor response was used for the primary analysis and these assessments were subsequently submitted to AstraZeneca for review by the response evaluation committee. Secondary efficacy endpoints included time to progression (TTP), duration of response (the time interval between the date of first documented objective tumor response until the date of PD or death), and disease control rate (DCR) for each dose of vandetanib. Time to progression was calculated from the date of randomization until the date of PD or death (in the absence of progression) and estimated using the Kaplan–Meier method. DCR was defined as confirmed complete response, PR, or stable disease (SD) ≥8 weeks.

Safety and Tolerability

Safety was assessed by monitoring for adverse events (AEs) and collecting laboratory data. All AEs were collected for up to 30 days after the last dose of vandetanib and were graded according to Common Terminology Criteria for Adverse Events (CTCAE, version 3). Unless otherwise clinically indicated, 12-lead electrocardiograms were performed twice at screening, weekly for the first 8 weeks of treatment, and then once every 4 weeks thereafter. Vandetanib treatment was interrupted following: a single QTc measurement ≥550 milliseconds; 2 consecutive QTc measurements ≥500 milliseconds but <550 milliseconds; an increase of ≥100 milliseconds from baseline; or an increase of ≥60 milliseconds from baseline QTc to a QTc value ≥460 milliseconds. Upon resolution of QTc prolongation, vandetanib treatment was recommenced at a reduced dose.

Pharmacokinetics

To investigate the pharmacokinetic (PK) profile of vandetanib, blood samples were collected on the same days as scheduled electrocardiogram measurements. Plasma concentrations of vandetanib were determined using reversed-phase liquid chromatography–mass spectrometry. The col-

lected data were related to a nonlinear mixed effects model to estimate population PK using NONMEM V (v 1.1).

Tumor Biomarkers

An exploratory objective of this study was to investigate how variations in copy number or mutational status of the *EGFR* gene affect tumor response in advanced NSCLC patients receiving vandetanib treatment. Tumor biopsy samples were obtained from consenting patients, formalin-fixed, and embedded in paraffin. Gene copy number was investigated by fluorescence in situ hybridization using the LSI EGFR SpectrumOrange/CEP 7 SpectrumGreen probe (Vysis, Abbott Laboratories, IL) according to a previously published method.[15] Tumor samples had a high *EGFR* gene copy number if there was high gene polysomy (≥4 *EGFR* gene copies in ≥40% of tumor cells) or gene amplification (presence of tight *EGFR* gene clusters, an *EGFR* gene to chromosome 7 ratio of ≥2, or ≥15 copies of the *EGFR* gene per tumor cell in ≥10% of analyzed cells).

EGFR mutations were analyzed by DNA sequencing of exons 19–21, and additionally by using the amplification refractory mutation system (ARMS) assay to detect the exon 21 L858R point mutation and the most common exon 19 deletion (del G2235–A2249).[16]

Plasma Biomarkers

Plasma samples were collected from patients at baseline, day 29, and day 57, and stored at −70°C. The concentrations of the following angiogenic markers were determined by colorimetric Sandwich ELISA (R&D Systems, Minneapolis, USA): VEGF (Cat. #DVE00), the soluble angiopoietin receptor Tie-2 (Cat. #DTE200), and VEGFR-2 (Cat. #DVR200).

RESULTS

Patient Characteristics

Fifty-three patients were recruited from eight centers in Japan between December 27, 2004, and September 30, 2005. All were randomized on this study and received study drug. Patient characteristics and baseline demographics were generally similar in the three arms, and the patient populations were considered to be appropriate for the dose-finding objectives of this study (Table 1). At the time of data cut-off (23 January 2006), 11 patients were ongoing; PD was the most common reason for discontinuation (n = 35). Other reasons for discontinuation were AEs (n = 6) and withdrawal of consent (n = 1).

Efficacy

The overall ORR was 13.2% (95% CI: 5.5–25.3%) (7 of 53 patients), and all 7 responders were PRs (Table 2). According to vandetanib dose received, the ORRs were 17.6% (95% CI: 3.8–43.4%) (3 of 17 patients; 100 mg), 5.6% (95% CI: 0.1–27.3%) (1 of 18 patients; 200 mg), and 16.7% (95% CI:3.6–41.4%) (3 of 18 patients; 300 mg). In all cases, the response evaluation committee assessment of tumor responses was similar to the investigator assessments. The characteristics of those patients who achieved a PR are described in Table 3. Secondary efficacy assessments are presented in Table 2 and Figure 2.

Safety

Overall, the most common AEs were rash, diarrhea, hypertension, and QTc prolongation (Table 4). In general, no major differences were observed in the incidences of

TABLE 1. Patient Demographic and Baseline Characteristics (Full Analysis Set)

	Vandetanib 100 mg/d (n = 17)	Vandetanib 200 mg/d (n = 18)	Vandetanib 300 mg/d (n = 18)	Total (n = 53)
Median age, yr (range)	58 (30–78)	61 (43–77)	61 (44–77)	60 (30–78)
Male (%)	11 (64.7)	12 (66.7)	11 (61.1)	34 (64.2)
Female (%)	6 (35.3)	6 (33.3)	7 (38.9)	19 (35.8)
Smoking history[a]				
No (%)	5 (29.4)	8 (44.4)	7 (38.9)	20 (37.7)
Yes (%)	12 (70.6)	10 (55.6)	11 (61.1)	33 (62.3)
WHO performance status 0/1/2	5/12/0	7/11/0	6/12/0	18/35/0
Previous chemotherapy				
One regimen (%)	13 (76.5)	9 (50.0)	14 (77.8)	36 (67.9)
Two regimens (%)	4 (23.5)	9 (50.0)	4 (22.2)	17 (32.1)
Staging (%)				
IIIB	2 (11.8)	3 (16.7)	1 (5.6)	6 (11.3)
IV	14 (82.4)	12 (66.7)	15 (83.3)	41 (77.4)
Recurrent	1 (5.9)	3 (16.7)	2 (11.1)	6 (11.3)
Histology (%)				
Squamous	5 (29.4)	6 (33.3)	4 (22.2)	15 (28.3)
Adenocarcinoma	11 (64.7)	12 (66.7)	12 (66.7)	35 (66.0)
Other	1 (5.9)	0	2 (11.1)	3 (5.7)
Brain metastasis at study entry (%)	4 (23.5)	3 (16.7)	5 (27.8)	12 (23.6)

[a] No, patients who have smoked <100 cigarettes in their lifetime; Yes, patients who have smoked >100 cigarettes in their lifetime.

TABLE 2. Efficacy Summary

	Vandetanib 100 mg/d (n = 17)	Vandetanib 200 mg/d (n = 18)	Vandetanib 300 mg/d (n = 18)
Primary efficacy assessment			
Best response (RECIST)			
Partial response, n (%)	3 (17.6)	1 (5.6)	3 (16.7)
Stable disease ≥8 wk, n (%)	5 (29.4)	6 (33.3)	8 (44.4)
Disease progression, n (%)	9 (52.9)	10 (55.6)	7 (38.9)
Not evaluable, n (%)	0	1 (5.6)	0
Secondary efficacy assessments			
Disease control ≥8 wk, n (%)	8 (47.1)	7 (38.9)	11 (61.1)
Duration of response (wk)			
Median (range)[a,b]	na	na	15.9 (7.3–20.1)
Time to progression (wk)			
Median (range)[a]	8.3 (4.0–40.7)	12.3 (0–40.3)	12.3 (1.4–32.7)
No. of events	12	13	13

na, not applicable; RECIST, Response Evaluation Criteria in Solid Tumors.
[a] Median estimated using the Kaplan–Meier method.
[b] This parameter could not be estimated in the 100 and 200 mg/d arms owing to the lack of progressions by the date of data cut-off.

TABLE 3. Characteristics of Patients Who Were Partial Responders

Treatment (initial dose)	Gender	Age (yr)	Smoking History[a]	Histology	Previous Chemotherapy Regimens	Time to PR (d)	Duration of Response (d)
100 mg	Male	65	Yes	Adenocarcinoma	1	28	204[b]
100 mg	Female	72	No	Adenocarcinoma	1	78	141[b]
100 mg	Male	52	No	Adenocarcinoma	1	143	141[b]
200 mg	Female	69	No	Adenocarcinoma	1	26	140[b]
300 mg[c]	Male	69	Yes	Adenocarcinoma	2	31	51
300 mg	Female	68	No	Adenocarcinoma	1	28	81[b]
300 mg	Female	55	No	Adenocarcinoma	1	82	141

[a] No, patients who have smoked <100 cigarettes in their lifetime; Yes, patients who have smoked >100 cigarettes in their lifetime.
[b] Censored on the day of last tumor evaluation due to absence of disease progression (response ongoing at data cut-off).
[c] Patient started study treatment with 300 mg and the treatment was stopped 29 d after the start due to QTc prolongation. The patient re-started at a reduced dose level (200 mg) 35 d after the start.

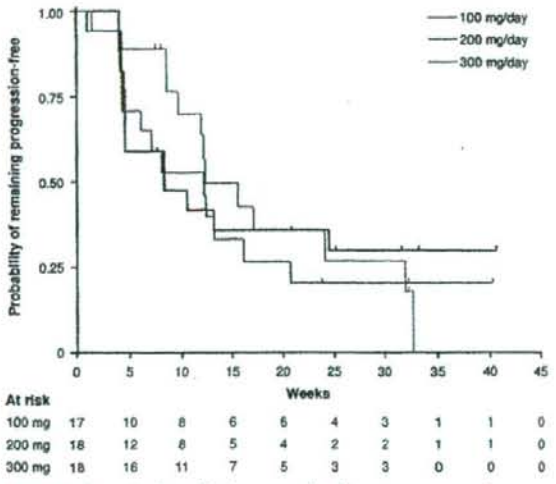

FIGURE 2. Kaplan–Meier curve for time to progression.

the common AEs across the three vandetanib arms, although the incidences of diarrhea, constipation, and abnormal hepatic function were numerically higher in the vandetanib 300 mg arm compared with the 100 or 200 mg arms. A dose-dependent increase in the incidence of CTC grade 3 and 4 events was observed; the incidence of these events in the 100, 200, and 300 mg dose arms were 29.4% (5 of 17 patients), 38.9% (7 of 18 patients), and 66.7% (12 of 18 patients), respectively. Of the 24 CTC grade 3 or 4 AEs considered by the investigator to be vandetanib-related, hypertension (100 mg, n = 4; 200 mg, n = 3; 300 mg, n = 3), and asymptomatic QTc prolongation (200 mg, n = 1; 300 mg, n = 1) were reported in more than one patient. Across the three dose levels, the AEs in this study were generally manageable with symptomatic treatment, dose interruption, or reduction.

Six patients discontinued vandetanib because of an AE considered by the investigator to be vandetanib-related: cryptogenic organizing pneumonia (COP), hepatic steatosis, and photosensitivity reaction (each n = 1, 200 mg arm); QTc prolon-

TABLE 4. Number of Patients With Most Commonly Reported Adverse Events (Occurring in ≥10% Across all Treatment Groups), Regardless of Causality

MedDRA Preferred Term[a]	Vandetanib 100 mg/d (n = 17)	Vandetanib 200 mg/d (n = 18)	Vandetanib 300 mg/d (n = 18)	Total (n = 53)
Rash (%)	10 (59)	9 (50)	9 (50)	28 (53)
CTC grade 3/4	0/0	1/0	0/0	1/0
Diarrhea (%)	8 (47.1)	8 (44)	11 (61)	27 (51)
CTC grade 3/4	0/0	1/0	1/0	2/0
Hypertension (%)	8 (47)	10 (56)	7 (39)	25 (47)
CTC grade 3/4	4/0	3/0	3/0	10/0
ECG QTc prolonged (%)	4 (24)	9 (50)	8 (44)	21 (40)
CTC grade 3/4	0/0	1/0	1/0	2/0
Photosensitivity reaction (%)	2 (12)	5 (28)	5 (28)	12 (23)
CTC grade 3/4	0/0	0/0	0/0	0/0
Nasopharyngitis (%)	3 (18)	4 (22)	4 (22)	11 (21)
CTC grade 3/4	0/0	0/0	0/0	0/0
Dry skin (%)	2 (12)	4 (22)	5 (28)	11 (21)
CTC grade 3/4	0/0	0/0	0/0	0/0
Nausea (%)	3 (18)	3 (17)	4 (22)	10 (19)
CTC grade 3/4	0/0	0/0	0/0	0/0
Constipation (%)	2 (12)	1 (6)	6 (33)	9 (17)
CTC grade 3/4	0/0	0/0	0/0	0/0
Fatigue (%)	4 (24)	1 (6)	2 (11)	7 (13)
CTC grade 3/4	0/0	0/0	0/0	0/0
ECG QT prolonged (%)	1 (6)	2 (11)	4 (22)	7 (13)
CTC grade 3/4	0/0	0/0	0/0	0/0
Hepatic function abnormal (%)	1 (6)	1 (6)	4 (22)	6 (11)
CTC grade 3/4	0/0	0/0	1/0	1/0
Hematuria (%)	2 (12)	2 (12)	2 (12)	6 (11)
CTC grade 3/4	0/0	0/0	0/0	0/0

[a] MedDRA version 8.1.

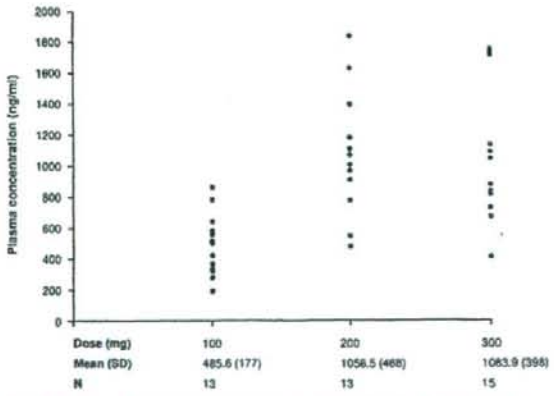

FIGURE 3. Observed maximum vandetanib plasma concentration at day 28. Patients who received dose reduction within the first 28 days were excluded.

gation, alanine aminotransferase increased, and erythema multiforme (each n = 1, 300 mg arm). Only COP was classed as a serious AE. Six patients had vandetanib dose reductions due to AEs (100 mg, n = 1; 200 mg, n = 1; 300 mg, n = 4).

Seven patients experienced eight respiratory-related events (COP, dyspnoea, interstitial lung disease [ILD], hypoxia, pneumonitis [all n = 1], and pneumonia [n = 3]). The incidence of these events in the three dose levels was 5.9% (1 of 17 patients; 100 mg), 11.1% (2 of 18 patients; 200 mg) and 22.2% (4 of 18 patients; 300 mg), respectively. Four of these events were considered to be related to vandetanib (COP, ILD, pneumonia [n = 2]). The ILD event was reported in a 64-year-old male patient in the 300 mg arm and resulted in patient death. This event was reported 8 days after vandetanib 300 mg was discontinued because of disease progression. No postmortem examination was performed and the investigator and a third-party physician considered the cause of death to be ILD.

All QTc prolongation was asymptomatic and manageable with dose interruption and/or reduction. The incidence of QTc prolongation was lower in the vandetanib 100 mg (24%) arm compared with the 200 mg (50%) and 300 mg (44%) arms. The mean change in QTc interval from baseline to week 3 (when maximum prolongation was observed) in the 100, 200, and 300 mg arms was +14 milliseconds (range, −25 to 29 milliseconds), +16.5 milliseconds (range, −36 to 49 milliseconds), and +27.6 milliseconds (range, 4 to 51 milliseconds), respectively. Protocol-defined QTc prolongation determined at the treatment site resulted in dose reduc-

come. In contrast, plasma levels of VEGFR-2 showed a trend to decrease over the same period, whereas plasma Tie-2 levels did not seem to change (Table 6). Baseline plasma VEGF levels appeared to be lower in patients who experienced clinical benefit following vandetanib treatment: PR (median 22.3 pg/ml, $n = 6$) and SD (median 37.0 pg/ml, $n = 16$) versus PD (median 63.7 pg/ml, $n = 21$). Patients with a low (below median) baseline plasma VEGF level had a longer TTP (median, 24.1 week) than those with a high (above median) baseline VEGF level (median, 8.3 weeks) (Figure 4). No clear relationship was apparent between baseline levels of plasma Tie-2 and VEGFR-2 and tumor response.

DISCUSSION

The primary objective of this phase IIa study was to assess the ORR to three doses of vandetanib (100, 200, and 300 mg/d) in Japanese patients with advanced or recurrent NSCLC. These doses of vandetanib were selected based on the outcomes of a Japanese phase 1 study where it was observed that vandetanib was well tolerated up to a dose of 300 mg and objective tumor responses were observed in 4 of 9 patients with NSCLC at doses of either 200 or 300 mg.[11]

In this study, objective tumor responses were observed at all three doses of vandetanib. The ORR in the 100, 200, and 300 mg arms was 17.6% (3 of 17 patients), 5.6% (1 of 18 patients), and 16.7% (3 of 18 patients), respectively. The DCR and TTP were similar across the three dose arms. It was noted that 50% (9 of 18) of the patients in the 200 mg arm had failed two previous chemotherapy regimens, compared with 23.5% (4 of 17 patients) and 22.2% (4 of 18 patients) in the 100 and 300 mg arms, respectively. It is possible that these differences contributed to the lower ORR observed in the 200 mg arm, although the number of patients in each dose arm was too small to allow any definitive conclusions to be made.

Vandetanib was well tolerated at 100, 200, and 300 mg dose levels in this study. Overall, AEs were generally mild and manageable with symptomatic treatment, dose interruption or reduction. In addition, the AE profile was consistent with that determined during phase I evaluation in patients with advanced solid tumors[10,11] and phase II monotherapy data in NSCLC.[12] Furthermore, the AE profile was also consistent with that reported previously for agents that inhibit the VEGFR[17,18] or EGFR[4,19] signaling pathways. In general, no apparent dose dependence was noted in the incidence of the common AEs in this study except for asymptomatic QTc prolongation (24%, 56%, and 44% for the 100, 200, and 300 mg dose arms, respectively), an event that was manageable by dose interruption/reduction.

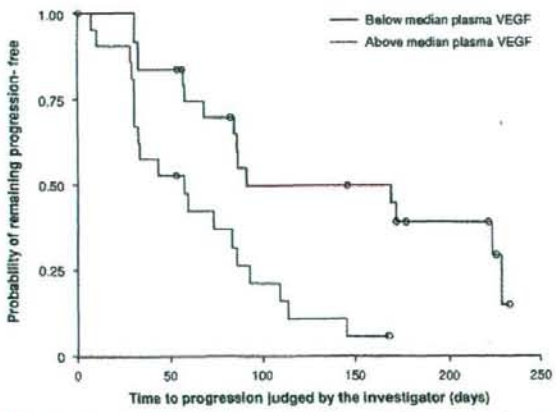

FIGURE 4. Kaplan–Meier curve of low (below median) versus high (above median) baseline plasma VEGF and time to progression.

A notable feature of this study, and the phase II program for vandetanib in NSCLC, is that patients with squamous cell histology or stable brain metastases were permitted to enter the trials. Both of these factors have been associated with an increased risk of bleeding, including severe life-threatening hemoptysis in NSCLC patients with squamous histology in a randomized phase II study of bevacizumab with carboplatin and paclitaxel.[20] These events have also been reported with other inhibitors of VEGF/VEGFR signaling, such as sunitinib and sorafenib.[17,18] Importantly, no CNS hemorrhage AEs or hemoptysis attributable to vandetanib were reported in this study.

The PK profile in this NSCLC patient population was consistent with that seen previously during Phase I evaluation in Japanese and USA/Australian patients with a range of solid tumors.[10,11]

In patients with NSCLC, specific *EGFR* mutations are associated with increased sensitivity to EGFR tyrosine kinase inhibitors,[21,22] and a better survival outcome with gefitinib has been shown to correlate with high *EGFR* gene copy number.[23] In this study, an exploratory analysis of tumor samples for amplification of *EGFR* gene copy number and somatic mutations of the *EGFR* gene revealed no clear relationship between *EGFR* mutation or gene amplification status and clinical outcome in patients receiving vandetanib. The *EGFR* mutation frequency of 4% (1 of 27 patients) is lower than that previously reported,[24,25] and further studies are needed to evaluate *EGFR* mutation status as a possible predictive marker for vandetanib therapy in advanced NSCLC.

In addition to *EGFR* mutation/amplification status, plasma profiling of cytokines and angiogenic factors may be a feasible approach for identifying blood-based prognostic and activity markers for therapies in NSCLC. Preliminary analysis of plasma concentrations of the angiogenesis markers VEGF and VEGFR-2 in the present study revealed that patients with PR or SD were more likely to have low baseline levels of VEGF than those with PD. It has been shown previously that low pretreatment levels of circulating VEGF correlated with a good response to gefitinib treatment in patients with NSCLC.[26] The significance of the relationship between these biomarkers and clinical outcome requires further investigation.

In conclusion, vandetanib monotherapy (100–300 mg/d) demonstrated antitumor activity with an acceptable safety and tolerability profile in Japanese patients with advanced NSCLC. Based only on this study, there is no com-

pelling evidence to identify the optimal dose of vandetanib monotherapy in this population of patients; further investigation of vandetanib doses in the range 100 to 300 mg is warranted in Japanese patients with advanced NSCLC. Other randomized phase II studies of vandetanib in advanced NSCLC have demonstrated improvements in progression-free survival with vandetanib 300 mg as a monotherapy versus gefitinib[12] and with the combination of vandetanib 100 mg and docetaxel.[14] Phase III evaluation of vandetanib in a broad population of patients, both as monotherapy at 300 mg (versus placebo in patients previously treated with anti-EGFR therapy [ZEPHYR]; versus erlotinib [ZEST]) and at 100 mg in combination with docetaxel (ZODIAC) or pemetrexed (ZEAL), has been initiated in global trials.

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Importance of *UDP-glucuronosyltransferase 1A1*6* for irinotecan toxicities in Japanese cancer patients

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Abstract

Recent pharmacogenetic studies on irinotecan have revealed the impact of *UDP glucuronosyltransferase (UGT) 1A1*28* on severe irinotecan toxicities. Although the clinical role of *UGT1A1*6*, which is specifically detected in East Asian patients, in irinotecan toxicities is suggested, clear evidence remains limited. To examine the impact of *6, the association of *UGT1A1* genotypes with severe irinotecan toxicities was retrospectively investigated in Japanese cancer patients. A significant *6-dependent increase in the incidence of grade 3 or 4 neutropenia was observed in 49 patients on irinotecan monotherapy ($p = 0.012$). This study further clarifies the clinical importance of *6 in irinotecan therapy in East Asians.

Keywords: UGT1A1; Pharmacogenetics; Irinotecan; SN-38

1. Introduction

Irinotecan, an anticancer prodrug, is widely applied for a broad range of carcinomas, including colorectal and lung cancers. The active metabolite, SN-38 (7-ethyl-10-hydroxycamptothecin), a topoisomerase I inhibitor, is generated by hydrolysis of the parent compound by carboxylesterases [1]. SN-38 is subsequently glucuronidated by uridine diphosphate glucuronosyltransferase 1As (UGT1As) such as 1A1, 1A7, 1A9 and 1A10, to form the inactive metabolite, SN-38 glucuronide (SN-38G) [2–5]. Among the UGT

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isoforms, UGT1A1 is thought to be a predominant contributor to SN-38G formation [2,6]. The dose-limiting toxicities in irinotecan therapy are severe diarrhea and leucopenia [7], and lowered UGT activity is well correlated with severe irinotecan toxicities [8]. Since Ando et al. first reported the significant relevance of *UGT1A1*28* – a repeat polymorphism in the TATA box (−40_−39insTA) – to severe neutropenia/diarrhea [9], a number of clinical studies, primarily conducted in Caucasian patients, have shown associations between *UGT1A1*28* and lowered SN-38G formation or severe neutropenia/diarrhea [10–13]. Based on these findings, the Food and Drug Administration (FDA) of the United States approved a revision of the label for Camptosar (irinotecan HCl) (NDA 20-571/S-024/S-027/S-028), recommending "a reduction in the starting dose by at least one level of irinotecan for the *UGT1A1*28* homozygous patients". Subsequently, the clinical application of *UGT1A1*28* testing was put into practice for irinotecan therapy in the United States.

To implement personalized irinotecan therapy in Asian countries, the racial differences in *UGT1A1* polymorphisms among Caucasians, African-Americans, and Asians must be taken into consideration [14]. For East Asians, the frequency of **28* is one third of that of Caucasians or African-Americans, and another low-activity allele **6* [211G>A(G71R)], which is not detected in Caucasians or African-Americans, shows the same frequency as the **28* allele. Clinical studies in Japanese cancer patients have demonstrated that significantly low area under concentration-time curve (AUC) ratios of SN-38G to SN-38 are observed in patients having **6* and/or **28* [15–17], suggesting the necessity of typing **6* in addition to **28*. A recent report on Korean lung cancer patients who received a combination therapy of irinotecan and cisplatin, showed a significant association of **6* homozygotes with severe neutropenia [18]. However, data on the role of **6* in irinotecan toxicities is still limited in terms of the various irinotecan-containing regimens. In the first study by Ando et al. on Japanese cancer patients, the association of **6* with irinotecan toxicities was not evident, but a possible enhancement of **28*-related toxicities by **6* was suggested [9]. Other studies in Japanese patients showed an additive effect of **6* on the lowered UGT activity by **28* [15–17]. A significant association of the genetic marker "**6 or *28*" with severe neutropenia was also shown in our previous study, but due to a lack of **6* homozygotes in our patient population, the effect of **6* alone was not confirmed [17].

In this study, to further demonstrate the clinical importance of **6* alone, *UGT1A1* genotypes were determined using DNA extracted from paraffin-embedded specimens (non-cancerous tissues) from 75 Japanese cancer patients by the pyrosequencing method [19,20], and the associations between *UGT1A1* genotype and severe irinotecan toxicities and serum total bilirubin levels were retrospectively analyzed.

2. Materials and methods

2.1. Patients and irinotecan treatment

In a post-marketing surveillance study conducted by Daiichi Pharmaceutical Co., Ltd. (currently Daiichi Sankyo Co., Ltd., Tokyo, Japan), irinotecan was prescribed to 297 patients with various types of cancers from 1995 to 2000 at the National Cancer Center Hospital. The patients were selected through standard clinical practice according to the drug label for indications and contraindications. Methanol-fixed, paraffin-embedded archival tissue specimens, which were necessary for high-quality extraction of DNA greater than 2 kb in size [21], were available for 75 of the 297 patients and were analyzed in this study. Irinotecan was administered by intravenous 30-min infusion as a single agent or in combination chemotherapy at a dose of 60 mg/m^2 (weekly or biweekly), 100 mg/m^2 (biweekly), or 150 mg/m^2 (biweekly). Profiles of the patients in this study, including cancer type, treatment history, and regimens, are summarized in Table 1. The pre-treatment levels of serum total bilirubin were determined by a kit (VL T-BIL, Azwell Inc., Osaka, Japan) according to an enzymatic method using bilirubin oxidase [22]. Toxicities were monitored during irinotecan therapy and graded according to the Common Toxicity Criteria version 2 of the National Cancer Institute.

Because the samples in this study were residual specimens remaining after histopathological diagnosis in the hospital and not collected specifically for research purposes, the samples and their clinical information were anonymized in an unlinkable fashion according to the Ethics Guidelines for Human Genome/Gene Analysis Research by the Ministry of Education, Culture, Sports, Science and Technology, Ministry of Health, Labour and Welfare, and Ministry of Economy, Trade and Industry of Japan. This study was approved by the ethics committees of the National Cancer Center and the National Institute of Health Sciences.

2.2. DNA extraction from paraffin-embedded tissue sections and genotyping of UGT1A1 polymorphisms

Three sections (20 μm of pathologically normal tissues around tumors) were deparaffinized twice by treat-

Table 1
Profiles of cancer patients in this study

		No. of patients
Patients genotyped (Male/female)		75 (51/24)
Age		
Mean/range (y)	50.7/34–75	
Performance Status[a]		
	0/1/2	18/48/8
Previous treatment		
Surgery[a]	+/−	71/3
Chemotherapy[b]	+/−	63/10
Radiotherapy[b]	+/−	9/64
Combination therapy and tumor type [dose of irinotecan (mg/m^2)/(w or 2w)[c]]		
Irinotecan monotherapy	Lung (60/w or 100/2w)	4
	Stomach (100/2w or 150/2w)	5
	Colon (100/2w or 150/2w)	40
With cisplatin	Lung (60/w or 100/2w)	4
	Stomach (60/2w)	11
With mitomycin C (MMC)	Stomach (150/2w)	8
	Breast (120/2w)	1
With 5-fluorouracil (5-FU)	Colon (150/2w)	2
Available data on serum bilirubin levels		37

[a] Data from one patient is lacking.
[b] Data from two patients are lacking.
[c] Weekly or biweekly.

ment with 1.5 ml of xylene at room temperature. After centrifugations, the residual pellet was then washed twice with 1.5 ml of ethanol. Finally, the pellet was dried at 37 °C for 15 min. DNA extraction was performed using a QIAamp tissue kit (QIAGEN K.K., Tokyo, Japan) according to the manufacturer's instructions with some modifications. Briefly, 540 μl of ATL lysis buffer and 60 μl of proteinase K (Qiagen) were added to each pellet, mixed thoroughly, and incubated at 56 °C for 3 h with a rotator. Any remaining tissue debris was removed by centrifugation, and the resulting supernatant was used for the extraction. Twelve microliters of RNase A (100 mg/ml) was added to the supernatant and incubated for 2 min at room temperature. Next, 600 μl of buffer AL was added and mixed thoroughly, and the mixture was incubated at 70 °C for 10 min. Six-hundred microliters of ethanol was added to the solution and mixed well, followed by extraction of DNA using a Qiagen DNA extraction column. The DNA was eluted in a final elution volume of 150 μl. The yield was determined using a NanoDrop spectrophotometer (NanoDrop Technology, Inc, Rockland, DE, USA) and the size of the extracted DNA was checked by agarose gel electrophoresis.

Genotyping of *UGT1A1*6* (211G>A, G71R), **28* (−364C>T, which is perfectly linked with −40_−39insTA in Japanese), and **60* (−3279T>G) were performed by pyrosequencing as described previously [19,20].

2.3. Association analysis and statistics

For association analysis, we focused on incidences of severe diarrhea and neutropenia (grade 3 or greater) observed during irinotecan-therapy. The incidence of severe diarrhea was very low, and the incidence of neutropenia was higher in combination therapy. Therefore, the association of neutropenia with *UGT1A1* genotypes was primarily evaluated in 49 patients with irinotecan monotherapy. As a parameter for in vivo UGT1A1 activity, serum total bilirubin levels taken at baseline from 37 patients were also used.

Statistical analysis for evaluation of the relationship between *UGT1A1* genotypes and severe neutropenia was performed using the chi-square test for trend using Prism version 4.0 (GraphPad Prism Software Inc., San Diego, CA). The gene-dose effect of the genetic marker "**6 or *28*" on serum total bilirubin levels was analyzed using the Jonckheere–Terpstra (JT) test in the SAS system (version 5.0, SAS Institute, Inc., Cary, NC). The *P*-value of 0.05 (two-tailed) was set as a significant level. Multivariate logistic regression analysis on neutropenia (grade 3 or greater) was performed using JMP software (version 6.0.0, SAS Institute, Inc., Cary, NC), including variables for age, sex, body surface area, performance status, concomitant disease, history of adverse reaction, irinotecan dosage, dosing interval, and *UGT1A1* genotypes. The variables in the final model for neutropenia were chosen using the forward and backward stepwise procedure at the significance level of 0.1.

3. Results

3.1. UGT1A1 diplotypes/haplotypes

The diplotypes and haplotypes (**1, *60, *6* and **28*) of *UGT1A1* exon 1 were analyzed in 75 Japanese cancer patients (Table 1) and their frequencies were summarized (Table 2). The haplotypes were assigned according to our previous definition [15]. It should be noted that the **60* haplotype does not harbor the **28* allele (−40_−39insTA), but most of the **28* haplotype does harbor the **60* allele (−3279T>G). In this study, the **28* homozygote was not present, and the frequency of haplotype **28* (0.113) was slightly lower than that found in our previous study (0.138) [17]. In contrast, the frequency of haplotype **6* (0.213) was higher than that found in the previous study (0.167) [17].

Table 2
Frequencies of *UGT1A1* diplotypes (A) and haplotypes (B) for cancer patients in this study

		Frequency
(A) Diplotype	No. of patients (N = 75)	
**1/*1*	21	0.280
**1/*60*	9	0.120
**60/*60*	2	0.027
**6/*1*	14	0.187
**6/*60*	8	0.107
**6/*6*	4	0.053
**28/*1*	12	0.160
**28/*60*	3	0.040
**28/*6*	2	0.027
**28/*28*	0	0.000
(B) Haplotype[a]	No. of chromosomes (N = 150)	
**1*	77	0.513
**60*	24	0.160
**6*	32	0.213
**28*	17	0.113

[a] Haplotype definition follows the previous report [15]; **60*, −3279T>G without −40_−39insTA; **6*, 211G>A(G71R); **28*, −40_−39insTA.

3.2. Association of UGT1A1 genotypes with serum total bilirubin levels

Serum total bilirubin levels at baseline, a parameter of in vivo UGT1A1 activity, were available from 37 patients (treated by various regimens), and we analyzed their association with *UGT1A1* genotypes (Fig. 1). The median values of total bilirubin in **60/*1*, **28/*1* and **6/*1* heterozygotes were not significantly different from that of the wild type (**1/*1*). Higher median values were observed for the **6* homozygotes (**6/*6*) and the double heterozygotes of **6* and **28* (**6/*28*) than that of the wild type (**1/*1*), with increases of 1.9-fold and 2.2-fold, respectively. Since **6* and **28* are mutually independent and their reducing effects on UGT activity are equivalent [15,17], diplotypes were classified by the presence of "**6 or *28*" (indicated by "+" in Fig. 1). As shown in Fig. 1, a significant "**6 or *28*"-dependent increase in total bilirubin levels was observed ($p = 0.0088$, Jonckheere–Terpstra test).

3.3. Severe toxicities observed in this study

Incidences of severe diarrhea and neutropenia (grade 3 or greater) are shown in Table 3 for each irinotecan-containing regimen. Grade 3 diarrhea was observed in only 4 of the 75 subjects, and since the incidence of diarrhea was low (5.3%), an association analysis on diarrhea was not conducted. Regarding neutropenia, 26 patients experienced grade 3 or 4 neutropenia. Of these 26 patients, 90% experienced neutropenia within 2 months after starting irinotecan-therapy, and 70% within 2 weeks. Significant differences in neutropenia incidences were observed among the regimens used, and considerably high incidences were observed in the combination therapies. Accordingly, association of the *UGT1A1* genotypes with severe neutropenia was analyzed primarily in the patients who received irinotecan-monotherapy.

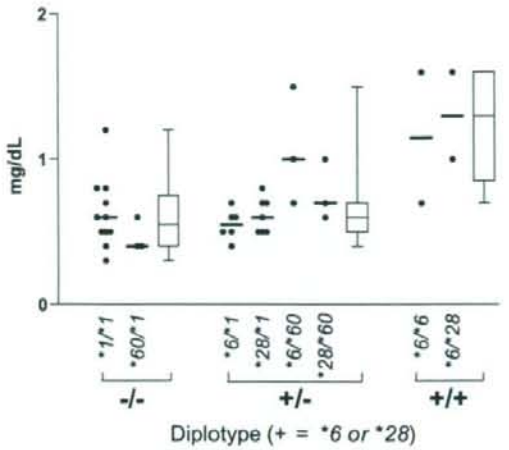

Fig. 1. Effects of *UGT1A1* genotypes on serum total bilirubin levels at baseline in Japanese cancer patients ($N = 37$). Each point represents a patient, and the median value of each diplotype is shown with a bar. All diplotypes are classified into −/−, +/−, and +/+ by the genetic marker, "*UGT1A1*6 or *28*", indicated by "+", and their distributions are shown by a box representing the 25–75 percentiles with a bar at the median and lines representing the highest and lowest values. A significant "**6 or *28*"-dependent increase in total bilirubin levels was observed ($p = 0.0088$, Jonckheere–Terpstra test).

Table 3
Severe toxicities observed in Japanese cancer patients

Treatment	Diarrhea[a]/total (%)	Neutropenia[b]/total (%)
Total patients	4/75 (5.3)	26/75 (34.7)
Irinotecan alone	1/49 (2.0)	6/49 (12.2)
With CDDP	2/15 (13.3)	11/15 (73.3)
With MMC	1/9 (11.1)	8/9 (88.9)
With 5-FU	0/2 (0.0)	1/2 (50.0)
P-value[c]	NS	<0.0001

[a] Grade 3.
[b] Grade 3 or 4.
[c] Chi-squre test.

3.4. Association of UGT1A1 genotypes with neutropenia

Since significant associations of *UGT1A1*6* and **28* with increased total bilirubin levels (decreased UGT-activity) were once again confirmed in this study, we assessed the clinical relevance of these haplotypes, focusing on the effect of **6* on severe neutropenia. In the 49

patients who received irinotecan monotherapy, the incidence of grade 3 or 4 neutropenia was *6-dependently increased ($p = 0.012$ in the chi-square test for trend). Namely, incidences of severe neutropenia in the *6 heterozygotes (*6/*1,*6/*60, and *6/*28) and homozygotes (*6/*6) were 2.3-fold and 15-fold higher, respectively, than that seen in the non-*6 bearing patients (*1/*1, *60/*1, *28/*1, and *28/*60) (Table 4). In this study, no *28 heterozygotes (*28/*1 and *28/*60) experienced any severe neutropenia, and there were no *28 homozygotes enrolled. Therefore, the effect of *28 could not be determined. For the *60-bearing patients without *6 or *28 (only heterozygote, *60/*1), one patient among six experienced severe neutropenia, and no significant *60-dependent increase was observed (data not shown). Although no statistically significant association of the *28 heterozygotes with severe neutropenia was confirmed in this study, the incidence of discontinuation of irinotecan monotherapy was higher in the *28-bearing patients (91%, $N = 11$) than that in the non-*28 subjects (79%, N = 38), while *60- or *6-dependent increased discontinuation rates were not found (data not shown). For the patients with cisplatin-combination therapy, a higher incidence of severe neutropenia was observed in the *6-bearing patients (*6/*1, *6/*60, and *6/*6) (100%, $N = 3$) than that in the non-*6 bearing subjects (*1/*1, *60/*1, *60/*60, and *28/*1) (66.7%, $N = 12$).

3.5. Multivariate analysis of neutropenia

In order to further clarify the clinical impact of *6 on irinotecan toxicities, multivariate logistic regression analysis on grade 3 or 4 neutropenia was conducted using variables, including *UGT1A1* genotypes and patient background factors, described in Section 2. The final model revealed a significant association of *6 with the incidence of grade 3 or 4 neutropenia at an odds ratio of 5.87 (Table 5).

4. Discussion

The clinical application of the genetic test for *UGT1A1*28* prior to irinotecan therapy has been in practice in the United States since 2005, which was based on cumulative evidence supporting the significant association of *28 with severe irinotecan toxicity [9–13]. Most of the evidence was obtained in Caucasian patients, where *28 is relatively frequent (30–40%) [14]. Although additive effects of another low activity allele, *6, which is specific for East Asians, has been also suggested [9,15–17], direct evidence in Japanese patients has remained limited. In this study, we clearly showed the significant correlation of *6 to grade 3 or 4 neutropenia in Japanese cancer patients who received irinotecan monotherapy. An increased incidence of severe neutropenia was also observed in the *6-bearing patients using cisplatin combination therapy. This finding is in accordance with a report on Korean lung cancer patients who received a combination therapy of irinotecan and cisplatin, which showed a significant association of *6 homozygotes with grade 4 neutropenia [18]. Since combination therapies using irinotecan may cause higher incidences of severe toxicities, the *UGT1A1* polymorphisms should be carefully considered in regimens that include irinotecan.

Since the alleles *6 and *28 are mutually independent [15] and their effects on the UGT activities were shown to be equivalent, the usefulness of the genetic marker "*6 or *28" for personalized irinotecan therapies has been suggested [17]. This was also supported in the current study, which showed a "*6 or *28"-dependent increase in serum total bilirubin levels (Fig. 1). Because of the low frequency of *28 without homozygotes among our subjects, the influence of *28 on toxicities was not clearly demonstrated, as in the case of the Korean patients where the allele frequency of *1A1*6* (23.5%) was much higher than that of *1A1*28* (7.3%) [18]. However, in the current study, the double heterozygotes of *6 and *28 (*6/*28) showed increases in serum total bilirubin levels (Fig. 1). Moreover, a higher incidence of severe neutropenia in the *6/*28 patients was observed, although the patient number was small ($N = 2$) (Table 4). This finding also indi-

Table 4
Association of *UGT1A1* genotypes with severe neutropenia (grade 3 or 4) in irinotecan monotherapy

Diplotype[b]	Neutropenia[a]/total (%)	Effect of *6 (%)	
–/–	1/20 (5.0)	non-*6/non-*6	(3.4)
*28/–	0/9 (0.0)		
*6/–	3/16 (18.8)	*6/non-*6	(22.2)
*6/*28	1/2 (50.0)		
*6/*6	1/2 (50.0)	*6/*6	(50.0)
P-value[c]		0.012	

[a] Grade 3 or 4.
[b] "–" represents "*1 or *60".
[c] Chi-square test for trend.

Table 5
Multivariate logistic regression analysis of severe neutropenia (grade 3 or 4) in irinotecan monotherapy

Variable	Coefficient	SE	*P*-value	Odds ratio	(95% Confidence limit)
*UGT1A1*6*	1.77	0.809	0.0289	5.87	(1.37–39.6)

$R^2 = 0.157$, Intercept = 3.15, $N = 49$.

cates the importance of "*6 or *28" in severe neutropenia, and in fact, a gene-dose effect of "*6 or *28" ($p = 0.04$ in the chi-square test for trend) and its significant contribution in multivariate analysis ($p = 0.0326$) were also confirmed (data not shown).

For the *60 haplotype (-3279T>G without -40_-39insTA), no association of *60 with severe neutropenia was observed in this study, which coincides with reports of other studies on Japanese cancer patients [17,23]. As for the *27 allele [686C>A(P229Q)], it was linked with the *28 allele and the haplotype was defined as the *28 subtype, *28c [15]. One *28c-heterozygous patient with irinotecan monotherapy showed no severe neutropenia, suggesting a small contribution of the *27 allele (data not shown).

In this study, the association between *UGT1A1* genotypes and antitumor activity was difficult to evaluate because of the small number of subjects stratified into each tumor type. Further clinical studies are needed to establish methods for selection of the appropriate regimen or dosage based on the *UGT1A1* genotypes, where a balance between toxicity and antitumor effect should be considered.

In conclusion, this study demonstrated the significant association of *UGT1A1*6* with severe irinotecan-mediated neutropenia. The current data also supported the usefulness of the genetic marker "*6 or *28" for personalized irinotecan therapy in Japanese, and likely East Asian, patients.

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ADVANCES IN DRUG DEVELOPMENT

Current Developments in Oncology Drug Research

Section Editor: Mark J. Ratain, MD

Population Differences in the Use of EGFR-targeted Agents

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H&O What do we currently know about epidermal growth factor receptor (EGFR)-targeted therapies and population differences?

NS EGFR-targeted therapies can largely be divided into 2 categories: EGFR small molecule tyrosine kinase inhibitors (TKIs) and antibodies.

First of all, we know that the response rates for EGFR TKIs such as gefitinib (Iressa, Astrazeneca) and erlotinib (Tarceva, Osi Phamaceuticals) are significantly higher in Asians, females, adenocarcinomas, and non-smokers. Survival rates are also better than in the total population. As for the toxicity profiles, incidences of pulmonary toxicities are higher in males, smokers, and squamous cell carcinomas. Within the Asian population, we currently know that the frequency of interstitial pneumonia is significantly higher in Japanese patients than in the Chinese and Korean population.

Secondly, there are the antibodies such as cetuximab (Erbitux, Imclone). This year at the American Society of Clinical Oncology meeting, the results from an interesting FLEX study—a randomized, multicenter, phase III investigation that compared cetuximab in combination with cisplatin/vinorelbine versus cisplatin/vinorelbine alone in advanced non-small cell lung cancer patients—were presented. There were very few data pertaining to the Asian population, but when the researchers divided data of the Caucasians and the Asians, the results seemed to be better in Caucasians. In the Asian population, there was no difference in survival rates; I think this is because the majority of Asian people receive small-molecule EGFR TKIs after antibody treatment, a factor that may confuse the survival results.

H&O How are these differences explained by EGFR and K-Ras mutation rates in certain populations?

NS About 30–40% of Asians are said to have an EGFR mutation. In Caucasians, the reported mutation rate is less than 10%. This corresponds with study results that show the same type of difference—a higher response and survival rates in the Asian population to EGFR inhibitor therapy—between the 2 populations. We currently do not know much about the mutation rates in other populations such as blacks, hispanics, etc., although it is said that in the hispanic population, the mutation rate seems to be very low.

In the European Society of Medical Oncology (ESMO) meeting this September, Professor Tony Mok from the Chinese University of Hong Kong presented results from the IRESSA Pan-Asia Study (IPASS), which clearly showed that EGFR mutation is related to response and survival.

The IPASS study, of which I was one of the co-workers, was an open label, randomized, parallel-group trial that tested gefitinib versus carboplatin/paclitaxel (carbo/paclitaxel) as first line treatment in a selected population of patients from Asia. It included 1,217 Asian people whose tumors were of adenocarcinoma histology, who had not

received prior chemotherapy, and who were non smokers or light smokers. Japanese people were about 20% of the participants; Chinese were about 30%; the rest were from other Asian countries. The aim of the trial was to demonstrate that gefitinib was non inferior to carbo/paclitaxel doublet chemotherapy.

Subjects were randomized (about 600 subjects in each arm) to gefitinib or carbo/paclitaxel (ie, standard chemotherapy). The primary endpoint was progression-free survival (PFS).

Results showed that the gefitinib group had superior PFS and higher tumor response compared with intravenous carbo/paclitaxel chemotherapy in the overall population. However, although the PFS in the gefitinib group was significantly better, we noticed that the 2 curves for gefitinib and carbo/paclitaxel crossed at 5–6 months. Interestingly, during the first 5–6 months, the carbo/paclitaxel group was doing better, but after that point, the gefitinib group showed better PFS. These were 2 very strange curves. Statistically, when we analyzed the differences using the Cox proportional hazard model, there was a significant difference between the 2 groups, overall favoring gefitinib. However, there is really no consensus as to whether crossed curves can be analyzed by the Cox proportional hazard model.

Also noteworthy was that among the 1,217 patients, about one-third were anaylized by biomarkers such as EGFR mutation, EGFR amplification, and EGFR expression. We found that in patients with EGFR mutation, gefitinib did significantly better than carbo/paclitaxel. However, in patients with the wild-type EGFR, the PFS of the carbo/paclitaxel group was significantly better than that of the gefitinib group. This was a very interesting observation.

As you know, patients who have an EGFR mutation do not have a K-Ras mutation, and vice versa. One might therefore speculate that, in a sense, K-Ras mutation is inversely associated with the efficacy of EGFR-targeted therapy, but the truth is that there is not enough data in lung cancer. In colon cancer, if the EGFR is mutated, anti-EGFR antibodies such as cetuximab are not effective. In lung cancer, we do not have much data mainly because K-Ras mutation rate is not very high.

H&O Have there been studies investigating the differences within the Asian population (ie, Japanese, Korean, Chinese, etc.)?

NS This is a difficult question because we have very few data. We do know that the mutation rates of the Japanese and Koreans are nearly the same—around 30–40%. At present, we do not have sufficient data on the mutation rates of the Chinese and other Asian countries, so we have not been able to make a complete comparison yet.

H&O What technology is there to detect EGFR mutation, and how reasonable is it to use it to predict EGFR TKI efficacy?

NS Some claim that other biomarkers such as EGFR amplification and fluorescence in situ hybridization (FISH) could also be indicators; but in my mind, they are not very reliable. I believe that EGFR mutation is the most reliable predictor we currently know. And reliability here depends on the number of samples; we need to get enough samples to analyze. How we detect mutation is a separate issue—a technical problem. I think that if we use copy numbers of the EGFR for amplification parameters, it would be reasonably reliable because it is very quantitatvie.

The problem with FISH results is that they contain 2 elements. FISH positive includes EGFR amplification and high polysomy. However, EGFR amplification is closely correlated to mutation whereas high polysomy does not show any correlation.

When studies include both, the end analysis may be very complicated. This is the case with the majority of the data from the University of Colorado Cancer Center or from Dr. Federico Cappuzzo at the Istituto Clinico Humanitas IRCCS in Italy, who sees FISH technology to be the best method for patient selection when the main endpoint is survival. But I think the mix of 2 different kinds of FISH data is very difficult for us to interpret. Even in the IPASS trial, analysis of survival based on FISH positivity showed a similar tendency but the analysis based on EGFR mutation was much more clear.

I also think that clinical factors such as nonsmoking, females, adenocarcinomas, etc. are related to these EGFR mutations. So at this point, I believe that EGFR mutation is most highly predictive. If patients have the mutation, nearly 80% of them will respond.

H&O Should EGFR TKIs be included in the initial therapy for patients with EGFR mutation?

NS This is a crucial question. And as was evident from my results at the ESMO meeting this year, we can conclude that for patients with EGFR mutation, the first choice of therapy could be gefitinib. For patients without EGFR mutation, chemotherapy should be chosen as the first choice of therapy. But, the IPASS data are for PFS and not overal survival (OS). We still need to wait for OS data, and it will take some time.

But I think the important thing is to focus on the primary endpoint of a clinical trial. If the primary endpoint is OS, it is rather easy for us to interpret the results. If

the primary endpoint is PFS or time to treatment failure (TTF), it is rather difficult to make hard conclusions. PFS and TTF are not that accurate, making them softer endpoints, which do not directly relate to patient benefit.

H&O What sort of studies do you think are necessary to investigate this topic further? Are there any ongoing that are noteworthy?

NS Right now, there are talks of 2 randomized Japanese trials: one by researchers at Tohoku University and the other by the West Japan Oncology Group (WJOG). The study designs are very similar; both are testing gefitinib versus platinum doublet in EGFR-mutated patients. The Tohoku group is testing carbo/paclitaxel, whereas the WJOG group is testing cisplatin plus docetaxel, for their chemotherapy arm. The primary endpoint is PFS. Both trials are currently accruing patients.

However, the IPASS data has heavily influenced these clinical trials because they have already shown that PFS in EGFR-mutated patients is significantly better in the gefitinib group than in the chemotherapy group. So the question whether to continue these 2 randomized trials has become an ethical one, and still remains unanswered.

J Lung Cancer 2008;7(1):1-8

Molecularly Targeted Therapy for Lung Cancer: Recent Topics

Many clinical trials of molecular target drugs have been done against advanced lung cancer, however, majority did not meet the primary endpoint. Positive studies of EGFR-TKI such as BR21 and Interest used unselected populations of non-small cell lung cancer. It was quite difficult to explain why they were positive. In the present review, the difficulties of clinical trial design in molecular target drugs were discussed based on the differences of the magnitude of antitumor activity and the target tumor cell population between cytotoxic drugs and molecular target therapy. **(J Lung Cancer 2008;7(1):1-8)**

Key Words: Lung cancer, Molecular target therapy, EGFR-TKI, Clinical trial

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The therapeutic efficacy of cytocidal anticancer drugs for lung cancer has reached a plateau(1~4), and it is extremely important to develop of new therapeutic agents. However, the majority of clinical trials of molecularly targeted drugs for lung cancer have yielded negative data, and the only drugs currently approved anywhere in the world are the EGFR-TKIs such as gefitinib and erlotinib and the anti-VEGF antibody, bevacizumab. Historically, matrix metalloprotease inhibitors(5), PKC α inhibitors, Ras kinase inhibitors(6), bexarotene, trastuzumab(7), etc.(8), have all been assessed with the prolongation of survival by simultaneous or consecutive use with cytocidal anticancer agents, but only negative data have been obtained (Table 1).

Table 1. Molecular Target-based Therapy in Lung Cancer

Specific target-based drugs	Combination	Results
Gefitinib (EGFR)	Y	Negative
	N	Negative, vs placebo
	N	Negative in Japanese, vs DTX
		Positive in Global, vs DTX
Erlotinib (EGFR)	Y	Negative
	N	Positive, vs placebo
Cetuximab (EGFR)	Y	Positive
Lonafarnib (ras)	Y	Negative
Bexarotene (RXR)	Y	Negative
Affinitac (PKC α)	Y	Negative
Sorafenib (Raf, VEGF etc)	Y	Negative
Trastuzumab	Y	Negative
Cetuximab	Y	Negative
Environment specific target-based drugs	**Combination**	**Results**
MMPI (Marimastat, Prinomastat)	Y	Negative
Bevacizumab	Y	Positive

N: No, Y: Yes

EGFR-TKIs

EGFR-TKIs are molecularly targeted drugs that selectively modify molecular biological abnormalities of tumor cells themselves(9~12). The amazing antitumor effect of EGFR-TKIs in cases in which platinum-taxane therapy failed attracted interest(13~16), but it was difficult to demonstrate that they contributed to any survival benefit(17~20). Erlotinib is used as second-line and third-line chemotherapy in cases of platinum-