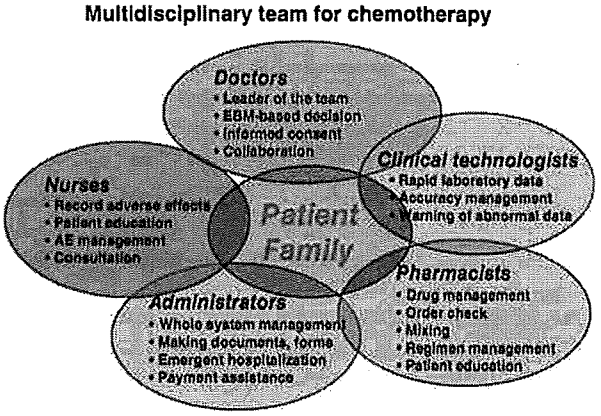

Fig. 5 Members of the multidisciplinary team for chemotherapy and their roles.

center), and medical collaboration officers in order to manage those adverse effects. The team made the clinical path for the initiation of bevacizumab therapy, the manual for managing the adverse effects of bevacizumab including consultation to gastrointestinal or respiratory surgeons, and made close liaison with cardiologists and neural surgeons in other hospitals. The team has revised the manual frequently, and solved the many problems associated with bevacizumab treatment.

In Japan, molecular target drugs such as sorafenib and sunitinib might be approved for RCC treatment in 2008. We are planning to make a new professional team consisting of medical oncologists, urological surgeons, nurses, pharmacists, and medical collaboration officers. The team will simulate the management of the severe adverse effects of these drugs, and make clinical paths and new manuals. We are also planning to start translational research such as a biomarker study.

In conclusion, in the area of urological malignancy, medical oncologists can participate in (i) some part of chemotherapy, (ii) care of complications with chemotherapy, (iii) experimental therapy such as phase I study, (iv) facilitating multidisciplinary care of patients, and (v) facilitating translational research.

Collaboration between the Japanese Society of Medical Oncology and the Japanese Urological Association for developing training systems for medical oncologists

Presenter

Hironobu Minami MD
Professor
Medical Oncology, Kobe University School of Medicine and Kobe University Hospital

It is well recognized that the quality of chemotherapy in oncology practice in Japan is lower than that in the United States or European countries. Patients with cancers are treated in an organ-specific medical system. Specifically, lung cancer is treated by chest physicians, gastrointestinal cancers by gastroenterologists or sometimes by surgeons, and genitourinary cancers by urologists. However, the organ-specific system often yields inadequate care. Patients with primary peritoneal adenocarcinoma presenting with ascites represent a subset of advanced cancers that are potentially curable by chemotherapy and surgical procedures for epithelial ovarian cancer. They often visit gastroenterologists with symptoms of abdominal fullness. Unfortunately, however, gastroenterologists often inadequately treat such patients with palliative chemotherapy for gastroenterological cancers without curative intent because they have undergone training for gastroenterological malignancies but not gynecological cancers. Similarly, patients with lung metastases from RCC sometimes visit thoracic oncologists who are not trained in immunotherapy, and patients are often inadequately treated.

Systemic chemotherapy for cancers should be performed by medical oncologists who have undergone a training program that includes all malignancies. However, training systems for medical oncologists including genitourinary cancers are currently under development in Japan. It is highly recommended that the JSMO and the JUA collaborate to establish such training systems for medical oncologists.

Reference

1 Akaza H. Report from the first Japanese Urological Association-Japanese Society of Medical Oncology joint conference, 2006: 'a step towards better collaboration between urologists and medical oncologists'. *Int. J. Urol.* 2007; **14**: 375–83.

Jpn J Clin Oncol 2008;38(5)339–346
doi:10.1093/jjco/hyn024

Efficacy and Safety of Pemetrexed in Combination with Cisplatin for Malignant Pleural Mesothelioma: A Phase I/II Study in Japanese Patients

Kazuhiko Nakagawa[1], Koichi Yamazaki[2], Hideo Kunitoh[3], Toyoaki Hida[4], Kenichi Gemba[5], Tetsu Shinkai[6], Yukito Ichinose[7], Susumu Adachi[8], Yoshihiro Nambu[9], Nagahiro Saijo[10] and Masahiro Fukuoka[1]

[1]Kinki University School of Medicine, Department of Medical Oncology, Osakasayama, Osaka, [2]Hokkaido University School of Medicine, First Department of Medicine, Sapporo, [3]National Cancer Center Hospital, Department of Internal Medicine and Thoracic Oncology, Tokyo, [4]Aichi Cancer Center Hospital, Department of Thoracic Oncology, Nagoya, [5]Okayama Rosai Hospital, Department of Respiratory Medicine, Okayama, [6]NHO Shikoku Cancer Center, Department of Medicine and Thoracic Oncology, Matsuyama, [7]National Kyushu Cancer Center, Department of Thoracic Oncology, Fukuoka, Japan, [8]Eli Lilly and company, Lilly Research Laboratories, Indianapolis, IN, USA, [9]Eli Lilly Japan K.K., Lilly Research Laboratories Japan, Kobe and [10]National Cancer Center Hospital East, Kashiwa, Chiba, Japan

Received October 3, 2007; accepted March 1, 2008; published online April 22, 2008

Background: Pemetrexed in combination with cisplatin (Pem/Cis) is used globally for the treatment of malignant pleural mesothelioma (MPM). This Phase I/II study was conducted to determine the recommended dose (RD) (Phase I) of Pem/Cis, and evaluate the efficacy and safety (Phase II) in Japanese MPM patients.
Methods: Key eligibility criteria were histologic diagnosis of MPM incurable by surgery, no prior chemotherapy, and a performance status 0–1. Under full vitamin supplementation, pemetrexed was intravenously administered on Day 1 of a 21-day cycle, followed by cisplatin. A cohort of six patients, starting from pemetrexed 500 mg/m^2 and cisplatin 75 mg/m^2 (Level 1), were studied in the dose-escalation Phase I (Step 1). The RD determined in Step 1 was carried forward into Phase II (Step 2). Planned number of patients treated with Pem/Cis was 18–38.
Results: In Step 1, 13 patients were enrolled: seven in Level 1 and six in Level −1 (pemetrexed 500 mg/m^2, cisplatin 60 mg/m^2). Two of six evaluable patients had dose-limiting toxicities (pneumonitis and neutropenia) in Level 1, establishing Level 1 as the RD. In Step 2, 12 patients were enrolled, for a total of 19 patients treated at the RD. Seven patients achieved a partial response among these patients, for a response rate of 36.8% (95% confidence interval: 16.3–61.6); overall survival was 7.3 months. One drug-related death occurred due to worsening of a pre-existing pneumonia. Common grade 3/4 toxicities were neutropenia and decreased-hemoglobin.
Conclusion: The Pem/Cis combination provides promising activity and an acceptable safety profile for chemonaive Japanese MPM patients with the same recommend dosage and schedule used in rest of the world.

Key words: cisplatin – mesothelioma – pemetrexed – phase I/II

INTRODUCTION

Malignant pleural mesothelioma (MPM) is a tumor derived from the mesothelium covering the surface of pleural membranes or from undifferentiated mesenchymal cells in connective tissue under the membranes. MPM is a locally invasive and aggressive tumor with a poor prognosis and a median survival time (MST) of ≈9–16 months (1).

MPM is known to be linked to asbestos exposure, and the incidence of this tumor is expected to increase in the next 10–20 years according to an estimation of asbestos consumption in

For reprints and all correspondence: Kazuhiko Nakagawa, Kinki University School of Medicine, Medical Oncology, 377-2 Ohnohigashi, Osakasayama 589-8511, Japan. E-mail: nakagawa@med.kindai.ac.jp

the world (9). Recently, the prevalence of MPM in Japan was widely recognized after uncovering the high incidence of MPM and MPM-related deaths in ex-workers of asbestos factories and in residents of the surrounding areas who may have been subject to non-occupational exposure to asbestos fibers.

Surgical resection offers local control of the tumor but its effect on survival remains unclear. In addition, application of radiation therapy is limited because of the diffuse extension of tumor spread. Regimens applied to lung cancer such as platinum-containing chemotherapy have been used for MPM in Japan; however, the efficacy outcomes of these therapies are not satisfactory. Therefore, effective systemic chemotherapy for MPM is clearly needed.

Pemetrexed is a novel antifolate (12) that inhibits three enzymes in folate metabolism: thymidylate synthase, dihydrofolate reductase and glycinamide ribonucleotide formyltransferase (11). Because of the multi-targeted profile of this compound, broad and preferable anti-tumor activity is expected. Pemetrexed has shown clinical activity in various tumors including mesotheliomas (6). A pivotal multicenter, randomized Phase III study of pemetrexed (500 mg/m^2) in combination with cisplatin (75 mg/m^2) versus cisplatin alone (cisplatin 75 mg/m^2) in patients with MPM who had no prior chemotherapy was conducted in 20 countries (not including Japan) (16). A total of 448 patients were randomized and treated in this study (226 treated by pemetrexed/cisplatin (Pem/Cis) and 222 treated by cisplatin). MST in the Pem/Cis arm was 12.1 months compared with 9.3 months in the cisplatin arm ($P = 0.020$, two-sided log rank test). This was the first confirmation of significant prolongation of survival for patients with MPM. On the basis of this evidence, the combination of pemetrexed and cisplatin was approved for the treatment of MPM in the USA in 2004. Since then, the combination therapy has been approved in more than 80 countries and regions for the treatment of MPM, and recognized as a standard care for MPM (8).

In 2005, we initiated a Phase I/II study of Pem/Cis therapy in Japanese patients with MPM who had no prior chemotherapy. The primary objectives of this study were to determine the clinically recommended dose (RD) of Pem/Cis therapy in the Phase I portion of the study (Step 1), and to examine tumor response of the combination therapy in the Phase II portion (Step 2). The secondary objectives included time-to-event efficacy outcomes [the duration of response, progression free survival (PFS), and overall survival time], 1-year survival rate, quality of life (QOL) assessments, pulmonary function tests and safety.

PATIENTS AND METHODS

Patient selection

Chemonaive patients with histological diagnosis of MPM, regardless of clinical stage and who were not candidates for curative surgery, were assessed for eligibility. Eligible patients needed to be 20–74 years old with a life expectancy $\geq$12 weeks and an Eastern Cooperative Oncology Group performance status (PS) 0 or 1. Patients were also required to have adequate organ functions: bone marrow reserve [platelets $\geq 100 \times 10^3/mm^3$, hemoglobin $\geq$9.0 g/dl, and absolute neutrophil count (ANC) $\geq 2.0 \times 10^3/mm^3$], hepatic function [bilirubin $\leq 1.5 \times$ upper limit of normal (ULN), aspartate/alanine transaminase (AST/ALT) $\leq 2.5 \times$ ULN, and serum albumin $\geq$2.5 g/dl], renal function (serum creatinine $\leq$ULN, and calculated creatinine clearance $\geq$45 ml/min using the Cockcroft and Gault formula), lung function (functional oxygen saturation [SpO_2] $\geq$92%) and normal electrocardiogram.

Patients were excluded from this study for active infection, symptomatic brain metastasis, a wide-spread diffuse shadow in the lung caused by interstitial pneumonitis diagnosed by chest X-ray, pregnancy, serious concomitant systemic disorders incompatible with the study, clinically significant effusions, Common Terminology Criteria for Adverse Events (CTCAEs) v3 grade $\geq$2 peripheral neuropathy, the inability to discontinue aspirin and other non-steroidal anti-inflammatory agents or the inability or unwillingness to take folate and vitamin B_{12} during the study.

This study was conducted in compliance with the guidelines of good clinical practice and the Declaration of Helsinki, and it was approved by the local institutional review boards. All patients gave written informed consent before study entry. The Efficacy and Safety Evaluation Committee (ESEC), an independent body, was consulted if any efficacy and safety issues arose in the study.

Study Design

This was a Phase I/II, multicenter, single-arm, open-label study, performed in two steps. The RD level established in Step 1 was carried forward in Step 2. Patients enrolled in Step 1 at the RD level could continue in Step 2 unless otherwise indicated. The planned number of patients in total of Steps 1 and 2 treated with Pem/Cis was 18–38 for examination of efficacy and safety profile. In Step 1, six patients were to be enrolled in each dose level. The lower number of the planned number of patients, 18, was set as the minimum number of patients needed to confirm that the response rate of the study drugs was significantly larger than the threshold rate of 10% at one-sided significant level 0.05 with $\geq$80% power.

Study Treatment

Pemetrexed was intravenously administered as a 10-min infusion on Day 1 of a 21-day cycle, followed by cisplatin administration intravenously as a 2-h infusion 30 min after pemetrexed administration. Patients were instructed to take a daily 1 g multivitamin containing 500 μg of folate beginning 1 week prior to Day 1 of Cycle 1 until study discontinuation. Vitamin B_{12} (1000 μg) was intramuscularly injected, starting 1 week prior to Day 1 of Cycle 1 and repeated every 9 weeks until study discontinuation. Patients remained on study unless they were discontinued, for instance, due to disease progression and unacceptable adverse events.

Determination of RD for Step 2

In Step 1 (Phase I), four escalating dose levels were planned: pemetrexed at 500 (Level 1), 700 (Level 2), 900 (Level 3) and 1000 mg/m^2 (Level 4) with cisplatin held at 75 mg/m^2. In addition, a lower dose level (Level −1) was planned at pemetrexed 500 mg/m^2 and a lower dose of cisplatin 60 mg/m^2 for a failure case of dose-escalation in Level 1. In the dose-escalation procedure, the starting dose of pemetrexed was set to be 500 mg/m^2 which is ca. 40% of the maximum tolerated dose (MTD) of pemetrexed monotherapy with folic acid and vitamin B_{12} supplementation determined in a Japanese Phase I study; the MTD and RD of pemetrexed were determined to be 1200 and 1000 mg/m^2, respectively (7). The percentage of the starting dose to the MTD was based on a guideline for Phase I/II study on anticancer drugs (10). For escalation of pemetrexed dose, a modified Fibonacci dose-escalation method was used (2). Dose level reduction or escalation depended on the incidence of dose-limiting toxicity (DLT) at a given dose level (Fig. 1). If two of six patients at Levels 1, 2 or 3 developed DLT, that dose level was considered the RD for Step 2 (Phase II) of the study, and then Step 2 was initiated. This was also the case for Level −1 or 4 if 0–2 patients developed DLT. If three or more patients developed DLT at a given dose level (except dose Level −1), the next lower dose level was considered the RD level for Step 2. If three or more patients had DLT at Level −1, a decision was made as to whether the study should be continued.

A DLT was defined as a toxicity occurring in Cycle 1 meeting one of the following criteria: any grade ≥3 non-hematologic toxicity (except nausea, vomiting, anorexia and fatigue), grade ≥2 peripheral neuropathy or hearing loss/impairment, grade ≥3 febrile neutropenia (<1000/mm^3 with ≥38.5°C), grade 4 leukopenia (<1000/mm^3) or neutropenia (<500/mm^3) lasting ≥3 days, thrombocytopenia (<25000/mm^3), or thrombocytopenia requiring platelet transfusion. A failure to start the second cycle by Day 29 due to toxicity was also considered a DLT. All toxicities were assessed according to CTCAE.

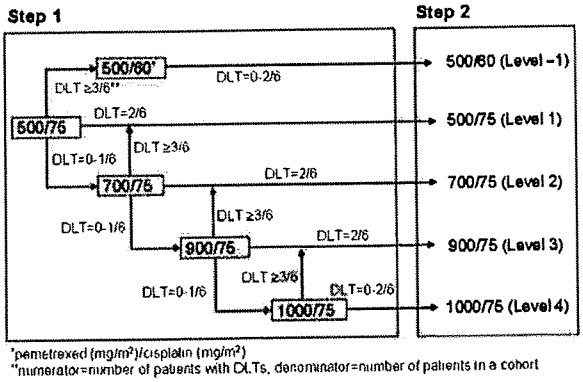

Figure 1. Scheme of dose-escalation Steps 1 and 2. DLT, dose-limiting toxicity.

Treatment Assessments

Anti-tumor Activity

Disease staging was assessed according to International Mesothelioma Interesting Group Tumor Node Metastasis (IMIG TNM) staging criteria (13). Within 28 days before the first treatment and approximately every 4 weeks after the first treatment, computer tomography or X-ray imaging of each lesion was performed. Tumor response was assessed using the modified Southwest Oncology Group (SWOG) criteria. Unidimensionally measurable lesions were defined as *Measurable disease*, and assessed objectively by the sum of the greatest diameters of them. Bidimensionally measurable lesions defined in the standard SWOG criteria (5) were assessed in the similar way. Best overall response selected from total overall response assessments was determined according to assessment of the Extramural Case Judgment Committee (E-CJC). Duration of response was measured as from the date of the first objective assessment of complete response (CR) or partial response (PR) until the date of the first assessment of progression of disease (PD). PFS was measured as from the registration date of Cycle 1 treatment until the first date of PD or death from any cause. Overall survival time was measured as from the registration date of Cycle 1 treatment until the date of death from any cause or until the last follow-up date in survival surveillance period.

QOL Assessments and Pulmonary Function Tests

QOL surveillance was employed using the following questionnaires: QOL questionnaire for cancer patients treated with anticancer drugs (QOL-ACD), and functional assessment of cancer therapy for lung cancer (FACT-L). These questionnaires were used on Day 1 of Cycles 1 and 2, and on 3 months after Day 1 of Cycle 1. QOL-ACD consists of four subscales (activity, physical condition, psychological condition and social relationships) and a total QOL scale (face scale) (4). The lung cancer subscale (LCS) score of FACT-L was used (3). As pulmonary function tests, forced vital capacity (FVC), forced expiratory volume in 1 s (FEV_1) and vital capacity (VC) were measured using a spirometer in the sitting position. All tests followed the Japanese Respiratory Function Test guidelines (14).

Safety

Adverse events were recorded throughout the study and after the last drug administration until signs of recovery were evident. Adverse events were evaluated according to treatment-emergent adverse events (TEAEs) definitions, and coded using the Medical Dictionary for Regulatory Activities (MedDRA v9.0). The severity (grade) of an adverse event was assessed according to CTCAE v3.

Statistical Analysis

The evaluation period of efficacy and safety in this study was defined as from the beginning of the study treatment to 5 months after the last patient began study treatment. For the

evaluations of overall survival time and 1-year survival rate, survival surveillance period was defined as from the beginning of the study treatment to 1 year after the last patient began study treatment. Patients who received the study drugs and complied with all inclusion/exclusion criteria were included in full analysis set (FAS). Patients who were treated with the RD level in Step 1 or 2 among FAS were included in efficacy analysis set for efficacy evaluation. Patients who received the study drugs at least once were included in safety analysis set for safety evaluation.

Assessment results of the best overall response by the E-CJC were used for efficacy analysis. Statistical tests based on binominal distribution were done to confirm that the response rate of the study drugs was significantly larger than the threshold rate of 10% at one-sided significant level 0.05. The threshold rate 10% was set on the basis of historical data on the response rate of cisplatin alone arm reported in other studies (15,16).

RESULTS

Patient Characteristics

From 2005 to 2006, a total of 25 Japanese patients with MPM were enrolled in Steps 1 and 2 at seven centers in Japan. All patients met the eligibility criteria and received study treatment; all were included in FAS. One patient was still receiving the study drug at the time of the efficacy and safety evaluations in this report.

Patient characteristics are summarized in Table 1. The majority of patients were male (22 patients, 88.0%). The median age was 61 years (range: 50–74 years). Most patients had a PS of 1 (18 patients, 72.0%) and clinical stage IV (21 patients, 84.0%). The predominant histologic subtype was epithelial in 64% of patients. Two demographic characteristics showed differences among dose levels. There were more patients with PS 0 in Level −1 (50.0%) than in Level 1 (21.1%). All six (100%) patients in Level −1 had the epithelial subtype versus 10 (52.6%) patients in Level 1.

Table 1. Patient characteristics

	Step 1 Level −1 ($n = 6$)	Level 1 ($n = 19$)	All treated ($n = 25$)
Gender			
Male	5	17	22
Female	1	2	3
Age			
Mean	61	61	61
SD	3.9	6.3	5.8
Med	61	59	61
Weight(kg)			
Mean	62.8	58.1	59.2
SD	8.51	11.19	10.65
Performance status prior to Cycle 1			
0	3	4	7
1	3	15	18
Histological subtype			
Epithelioid mesothelioma	6	10	16
Sarcomatoid mesothelioma	0	5	5
Biphasic mesothelioma	0	4	4
Other	0	0	0
Asbestos exposure			
Had no exposure	2	3	5
Had exposure	4	16	20
Stage of disease			
Ia	0	0	0
Ib	0	1	1
II	0	1	1
III	1	1	2
IV	5	16	21

Level 1: pemetrexed 500 mg/m^2 + cisplatin 75 mg/m^2
Level −1: pemetrexed 500 mg/m^2 + cisplatin 60 mg/m^2
SD, standard deviation.

Dose-Escalation, Dose-Limiting Toxicity and RD

One patient in Level 1 of Step 1 died on Day 14 of Cycle 1 due to exacerbation of pneumonia, respiratory failure (hypoxia) and disseminated intravascular coagulation (DIC). The ESEC evaluated the case of the early death. Since the patient had had the shadow of the lung detected by radiographic image prior to receiving study treatment, it was unlikely that the administration of pemetrexed was the primary cause of the pneumonia. The autopsy of this patient showed that interstitial changes in the lung were mild and the pathological diagnosis was an organizing pneumonia. The result of the autopsy was compatible with the clinical course and suggested that the direct cause of the death was not the drug-induced interstitial pneumonia but the exacerbation of infectious pneumonia, worsened by the study treatment. The case, therefore, was considered not appropriate for the DLT evaluation.

One patient was added in this dose level to assess the safety profile additionally. Among the six patients in Level 1 excluding the case inappropriate for the DLT evaluation, two patients showed DLTs: drug-induced pneumonitis in one patient and dose delay of Cycle 2 initiation due to decreased neutrophil count in the other. According to the protocol definition, Level 1 was determined to be an RD for the next phase (Fig. 1).

The ESEC, however, recommended examining the treatment at Level −1 (pemetrexed 500 mg/m^2 and cisplatin 60 mg/m^2) exploratively to accumulate more safety information. Accordingly, six patients were enrolled and treated at Level −1, and no DLTs were observed in this dose level.

Evaluating the data of these two levels together, the ESEC agreed to continue Step 2 carefully with the dose of Level 1. The sponsor decided to carry forward into Step 2 with

an RD of Level 1 (pemetrexed 500 mg/m^2 and cisplatin 75 mg/m^2). In Step 2, 12 patients were treated at Level 1.

Efficacy

Nineteen patients (7 in Step 1 and 12 in Step 2) in Level 1 were included in the efficacy analysis set and of 19 patients, seven patients had PR, five patients had stable disease (SD), six patients had PD and one patient was classified as not evaluated. An overall response rate (ORR) was 36.8% [95% confidence interval (CI): 16.3%–61.6%]. The 95% one-sided confidence lower limit was 18.8%, exceeding the threshold level of 10%. The six patients in Level −1 had PR; thus, the ORR for all 25 patients treated with the study drug reached 52.0% (13 total PR, 95% CI: 31.3%–72.2%).

The secondary efficacy variables were time-to-event outcomes (the duration of response, PFS and overall survival time), 1-year survival rate, QOL and pulmonary function test. The median duration of response was 5.2 months (95% CI: 4.3–7.3 months) for the seven responders in the efficacy analysis set (Table 2). The median duration of response for the six responders at Level −1 was again 5.2 months. For the efficacy analysis set, median PFS was 4.7 months (95% CI: 1.3–6.5 months) and MST was 7.3 months (95% CI: 4.6–14.2 months, Fig. 2) with 1-year survival rate of 36.8% (95% CI: 15.2%–58.5%). Median PFS for the six patients at Level −1 was 10.1 months. MST at Level −1 could not be calculated by Kaplan–Meier method. The 1-year survival rate of Level −1 (66.7%) was beyond 50%.

The QOL-ACD and FACT-L measures were used for QOL evaluation. There were no major changes from prior to Cycle 1 to 3 months after Cycle 1 treatment in the mean scores for the activity and physical condition subscales of QOL-ACD (Table 3); however, mean scores from prior to Cycle 1 to 3 months after Cycle 1 treatment for the psychological condition and social relationships subscales numerically increased. The mean LCS score of FACT-L did not change substantially from prior to Cycle 1 to 3 months after Cycle 1 treatment (data not shown). These score changes indicate that QOL of the patients was maintained without worsening from baseline. Pulmonary function was also maintained with no worsening from baseline observed in the pulmonary function tests (FEV_1, FVC and VC) in the efficacy analysis set (data not shown).

Safety

Of 25 patients of the safety analysis set, three died during the study period: one (Level 1, Step 1) from exacerbation of pneumonia as a pre-existing complication, respiratory failure, and DIC, as described earlier, and the other two (Step 2) due to study disease. Two patients experienced non-fatal serious adverse events (fever and aspiration pneumonia, respectively). A causal relationship between fever and the study drugs could not be ruled out, but the aspiration pneumonia was not considered related to study drugs. Adverse events leading to discontinuation from study treatment were observed in six patients: one patient at Level 1 and three patients at Level −1 in Step 1 and in two patients in Step 2. Adverse event leading to discontinuation in two or more patients was increased blood creatinine (two patients).

Table 2. Summary of time-to-event outcomes and 1-year survival rates

	Step 1 Level −1 (n = 6)	Level 1 (n = 19)	All treated (n = 25)
Duration of response (months)			
Responders	6	7	13
Med	5.2	5.2	5.2
(95% CI)	3.1 − *	4.3–7.3	4.3–7.3
Range	2.7–9.6	2.0–7.3	2.0–9.6
Censored (%)	50	14.3	30.8
Progression free survival (months)			
Med	10.1	4.7	4.8
(95% CI)	4.3 − *	1.3–6.5	2.5–7.1
Range	3.3–12.1	0.5–9.6	0.5–12.1
Censored (%)	50	10.5	20
Overall survival (months)			
Med	NA	7.3	9.2
(95% CI)	11.1 − *	4.6–14.2	5.8–14.4
Range	8.6–19.3	0.5–21.5	0.5–21.5
Censored (%)	66.7	21.1	32
1-year survival rate (%)			
	66.7	36.8	44.0
(95% CI)	28.9–100.0	15.2–58.5	24.5–63.5

*Not calculated. NA, not assessed.
Level 1: pemetrexed 500 mg/m^2 + cisplatin 75 mg/m^2.
Level −1: pemetrexed 500 mg/m^2 + cisplatin 60 mg/m^2.
CI, confidence interval.

Grade 3 or more laboratory TEAEs were observed in 16 patients: four patients at Level 1 and five patients at Level −1 in Step 1 and in seven patients in Step 2. Laboratory TEAEs observed in at least half of the 25 patients were decreased-hemoglobin, decreased red blood cell count, decreased neutrophil count, decreased white blood cell count, decreased lymphocyte count, increased blood urea and decreased body weight (Table 4). Grade 3 or more non-laboratory TEAEs were observed in eight patients: three patients at Level 1 and one patient at Level −1 in Step 1 and in four patients in Step 2. Non-laboratory TEAEs observed in at least half of the 25 patients were nausea, anorexia, vomiting and malaise. No major differences between Levels 1 and −1 (Step 1) in the incidence of TEAEs were noted.

For the 19 patients at Level 1, laboratory TEAEs of grade 3 or higher, possibly related to drug, and observed in at least two patients were decreased neutrophil count (seven patients, 36.8%), decreased hemoglobin (six patients, 31.6%), decreased white blood cell count (five patients, 26.3%), decreased lymphocyte count (five patients, 26.3%),

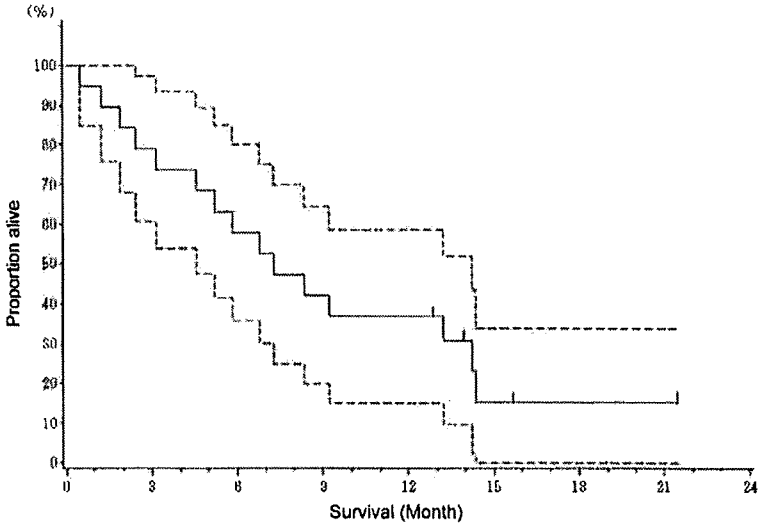

Figure 2. Kaplan–Meier plot of overall survival in the efficacy analysis set. Solid lines, overall survival; dotted lines, high and low limits of 95% confidence interval.

decreased platelet count (two patients, 10.5%) and decreased blood potassium (two patients, 10.5%). Non-laboratory adverse drug reactions of grade 3 or higher observed in at least two patients were vomiting (three patients, 15.8%), anorexia (three patients, 15.8%), nausea (two patients, 10.5%) and malaise (two patients, 10.5%). Adverse drug reactions of grade 3 or higher for the six patients in Level −1 were decreased neutrophil count (three patients), decreased-hemoglobin (two patients), decreased lymphocyte count (two patients) and decreased red blood cell count (one patient).

DISCUSSION

This Phase I/II study reports the first experience of the combination of pemetrexed and cisplatin therapy in Japanese patients. The RD of Pem/Cis combination therapy was established at pemetrexed 500 mg/m^2 and cisplatin 75 mg/m^2, with pemetrexed administration on Day 1 of each 21-day cycle followed by cisplatin, which is the same regimen used in worldwide for patients with MPM (16).

Of the 19 patients evaluable for efficacy at the RD level, there were PRs in seven patients, for an ORR of 36.8% (95% CI: 16.3%–61.6%). A pivotal Phase III study of the same regimen as that applied of the present study, yielded a response rate of 41.3% (95% CI: 34.8%–48.1%) in 225 patients (16). The response rates from both studies are comparable despite of the large difference in sample size.

The response rate of all the 25 treated patients was higher than the response rate for the 19 patients treated at the RD (52.0% versus 36.8%). This is due to the fact that all the six patients in Level −1 had PR. The excellent outcome observed in Level −1 may be attributed to differences between those patients who received the RD and those patients in Level −1 in the histological subtype of mesothelioma. All six patients in Level −1 had an epithelial subtype, which is known as a favorable prognostic factor, while only about half of the 19 patients at the RD had this subtype. In addition, the PS of the patients in Level −1 was better than the patients at RD.

A secondary efficacy endpoint MST showed 7.3 months in this study, shorter than that of the Pem/Cis arm in the Phase III study (12.1 months) (16). Although it would be difficult to compare MST of this study derived from a small sample size with the large Phase III study ($n = 226$), the discrepancy of survival between the two studies could be ascribed for the demographic characteristics of patients in both. There are less patients who had good prognostic factors in this study than in the Pem/Cis arm of the Phase III study: epithelial subtype: 52.6% versus 68.1%, a good PS: 21.1% (PS = 0) versus 51.8% (Karnofsky PS = 90/100) and clinical stage I/II: 8.0% versus 22.6% (16).

In this study, the most common adverse events (>50% of patients) were decreased-hemoglobin, erythropenia, neutropenia, leukopenia and lymphopenia for laboratory parameters, and nausea, anorexia, and vomiting for non-laboratory parameters. These hematologic and gastrointestinal events were similarly observed in the Pem/Cis arm of the pivotal Phase III study (16). No grade 3/4 febrile neutropenia toxicity which is a potentially life-threatening event was reported in our study. One death by pneumonitis was observed in this study; however, the patient was considered to have a pre-existing condition before initial treatment with study therapy. Adverse events observed in this study were predictable from safety profile observed in overseas trials and market experiences of pemetrexed and cisplatin combination therapy.

Table 3. Summary of QOL questionnaire for cancer patients treated with anticancer drugs (Level 1, $n = 19$)

Subscale	Measurement Point	n	Mean	SD	Min	Med	Max
Activity							
	Prior to Cycle1	19	62.9	25.35	20.0	60.0	100.0
	Prior to Cycle2	15	61.8	32.27	5.0	70.0	100.0
	Prior to Cycle3	14	69.6	21.79	20.0	75.0	95.0
	Cycle1 + 3M	11	60.5	32.13	5.0	70.0	100.0
Physical							
	Prior to Cycle1	19	64.7	22.33	15.0	70.0	100.0
	Prior to Cycle2	15	64.3	18.11	20.0	65.0	95.0
	Prior to Cycle3	14	66.2	18.33	30.0	70.0	85.0
	Cycle1 + 3M	11	61.4	21.46	35.0	60.0	95.0
Psychological							
	Prior to Cycle1	19	53.2	20.62	12.5	56.3	81.3
	Prior to Cycle2	15	59.6	24.87	12.5	62.5	100.0
	Prior to Cycle3	14	58.0	17.41	31.3	56.3	87.5
	Cycle1 + 3M	11	61.4	18.07	37.5	68.8	87.5
Social							
	Prior to Cycle1	19	32.9	21.56	5.0	25.0	75.0
	Prior to Cycle2	15	33.7	19.13	0.0	25.0	70.0
	Prior to Cycle3	14	43.6	19.94	10.0	42.5	85.0
	Cycle1 + 3M	11	36.4	22.59	10.0	30.0	85.0
Face scale							
	Prior to Cycle1	19	50.0	23.57	0.0	50.0	100.0
	Prior to Cycle2	14	55.4	24.37	0.0	50.0	100.0
	Prior to Cycle3	14	64.3	23.44	25.0	50.0	100.0
	Cycle1 + 3M	11	63.6	20.50	25.0	75.0	100.0

Level 1: pemetrexed 500 mg/m^2 + cisplatin 75 mg/m^2. M, months.
QOL, quality of life.

CONCLUSION

The RDs for the Pem/Cis combination are pemetrexed 500 mg/m^2 and cisplatin 75 mg/m^2, which is the same regimen used in worldwide for patients with MPM. The combination shows promising efficacy with an acceptable safety profile in Japanese patients with MPM.

On January 2007, Pem/Cis combination therapy was approved and launched for the treatment of patients with MPM in Japan. Intensive post-marketing surveillance in patients with MPM is ongoing.

Funding

This study has been supported and funded by Eli Lilly Japan K.K., Kobe, Japan.

Conflict of interest statement

S.A. and Y.N. are employed by the sponsor, Eli Lilly Japan K.K.; N.S. and M.F. are paid consultants to the sponsor.

Table 4. Summary of treatment-emergent adverse events (TEAEs) reported >25% patients

System organ class preferred term	Step 1 Level −1 ($n = 6$)	Level 1 ($n = 19$)	All treated ($n = 25$)
Patients with ≥1 TEAEs	6	19	25
Laboratory			
Hemoglobin decreased	6	18	24
Red blood cell count decreased	6	16	22
Neutrophil count decreased	5	16	21
White blood cell count decreased	5	15	20
Lymphocyte count decreased	5	12	17
Blood urea increased	5	11	16
Weight decreased	3	12	15
Blood albumin decreased	2	10	12
Platelet count decreased	4	8	12
Protein total decreased	3	9	12
Blood creatinine increased	4	7	11
Neutrophil count increased	2	8	10
White blood cell count increased	2	8	10
Blood sodium decreased	2	7	9
Alanine aminotransferase increased	1	7	8
Protein urine present	1	7	8
Aspartate aminotransferase increased	1	6	7
Blood magnesium decreased	2	5	7
Blood potassium decreased	0	7	7
Non-laboratory			
Nausea	6	18	24
Anorexia	6	16	22
Vomiting	3	15	18
Malaise	5	10	15
Constipation	3	9	12
Hiccups	3	5	8
Rash	2	6	8
Diarrhoea	1	6	7
Oedema	2	5	7
Pyrexia	2	5	7
Dysgeusia	3	4	7
Headache	1	6	7

Level 1: pemetrexed 500 mg/m^2 + cisplatin 75 mg/m^2
Level −1: pemetrexed 500 mg/m^2 + cisplatin 60 mg/m^2
MedDRA Ver 9.0.

References

1. British Thoracic Society Standards of Care Committee. Statement on malignant mesothelioma in the United Kingdom. *Thorax* 2001;56: 250–65.
2. Carter SK. Study design principles in the clinical evaluation of new drugs as developed by the chemotherapy programme of the National Cancer Institute. In: Staquet MJ, editor. The Design of Clinical Trials in Cancer Therapy. Brussels, Belgium: Futara Pub Co 1973;242–89.

3. Cella DF, Bonomi AE, Lloyd SR, Tulsky DS, Kaplan E, Bonomi P. Reliability and validity of the Functional Assessment of Cancer Therapy-Lung (FACT-L) quality of life instrument. *Lung Cancer* 1995;12:199–220.
4. Eguchi K, Kurihara M, Shimozuma K, Hotta T, Murakami M, Suzuki N, et al. Quality of life questionnaire for cancer patients treated with anticancer drugs. *Nippon Ganchiryo Gakkaishi* 1993;28:1140–4 (in Japanese).
5. Green S, Weiss GR. Southwest oncology group standard response criteria, endpoint definitions and toxicity criteria. *Invest New Drugs* 1992;10:239–53.
6. Hanauske AR, Chen V, Paoletti P, Niyikiza C. Pemetrexed disodium: a novel antifolate clinically active against multiple solid tumors. *Oncologist* 2001;6:363–73.
7. Nakagawa K, Kudoh S, Matsui K, Negoro S, Yamamoto N, Latz JE, et al. A phase 1 study of pemetrexed (LY231514) supplemented with folate and vitamin B_{12} in Japanese patients with solid tumors. *Br J Cancer* 2006;95:677–782.
8. Pass HI, Hahn SM, Vogelzang NJ, Carbone M. Benign and malignant mesothelioma-Chapter 36. In: DeVita VT Jr., Hellman S, Rosenberg SA, editors Cancer Principles and Practice of Oncology. 7th edn. Philadelphia: Lippincott Williams & Wilkins 2004;1687–715.
9. Peto J, Decarli A, La Vecchia C, Levi F, Negri E. The European mesothelioma epidemic. *Br J Cancer* 1999;79:666–72.
10. Saijo N and Study Group. Guideline for phase I/II study on anticancer drugs (Draft version). *Med Front (Saishin Igaku)* 2001;56:1515–41 (in Japanese).
11. Shih C, Habeck LL, Mendelsohn LG, Chen VJ, Schultz RM. Multiple folate enzyme inhibition: mechanism of a novel pyrrolopyrimidine-based antifolate LY231514 (MTA). *Adv Enzyme Regul* 1998;38:135–52.
12. Taylor EC, Patel HH. Synthesis of pyrazolo[3,4-d]pyrimidine analogues of the potent antitumor agent N-{4-[2-(2-amino-4(3H)-oxo-7H-pyrrolo[2,3-d]pyrimidin-5-yl)ethyl]benzoyl}-L-glutamic acid (LY231514). *Tetrahedron* 1992;48:8089–100.
13. The International Mesothelioma Interest Group. A proposed new international TNM staging system for malignant pleural mesothelioma. *Chest* 1995;108:1122–8.
14. The Japanese Respiratory Society. In: Clinical Pulmonary Functions Committee, editor. Guideline of Pulmonary Function Test. Tokyo: Medical Review 2004 (in Japanese).
15. Van Meerbeeck JP, Gaafar R, Manegold C, Van Klaveren RJ, Van Marck EA, Vincent M, et al. Randomized phase III study of cisplatin with or without raltitrexed in patients with malignant pleural mesothelioma: an intergroup study of the European Organisation for Research and Treatment of Cancer Lung Cancer Group and the National Cancer Institute of Canada. *J Clin Oncol* 2005;23:6881–9.
16. Vogelzang NJ, Rusthoven JJ, Symanowski J, Denham C, Kaukel E, Ruffie P, et al. Phase III study of pemetrexed in combination with cisplatin versus cisplatin alone in patients with malignant pleural mesothelioma. *J Clin Oncol* 2003;21:2636–44.

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

Katsuyuki Kiura, MD, PhD, Kazuhiko Nakagawa, MD, PhD,† Tetsu Shinkai, MD, PhD,‡ Kenji Eguchi, MD, PhD,§ Yuichiro Ohe, MD, PhD,‖ Nobuyuki Yamamoto, MD, PhD,¶ Masahiro Tsuboi, MD, PhD,# Soichiro Yokota, MD, PhD,** Takashi Seto, MD, PhD,†† Haiyi Jiang, MD,‡‡ Kazuto Nishio, MD, PhD,† Nagahiro Saijo, MD, PhD,§ and Masahiro Fukuoka, MD, PhD†*

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.

(*J Thorac Oncol.* 2008;3: 386–393)

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

*Okayama Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences and Okayama University Hospital, Okayama, Japan; †Kinki University School of Medicine, Osaka, Japan; ‡Shikoku Cancer Center, Ehime, Japan; §Tokai University Hospital, Kanagawa, Japan; ‖National Cancer Center Hospital, Tokyo, Japan; ¶Shizuoka Cancer Center, Shizuoka, Japan; #Tokyo Medical University Hospital, Tokyo, Japan; **Toneyama National Hospital, Osaka, Japan; ††Kyushu Cancer Center, Fukuoka, Japan; ‡‡AstraZeneca KK, Osaka, Japan; and the §§National Cancer Center Hospital East, Chiba, Japan.

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

Address for correspondence: Katsuyuki Kiura, MD, PhD, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences and Okayama University Hospital, 2-5-1Shikata-cho, Okayama 700-8558, Japan; E-mail: kkiura@md.okayama-u.ac.jp

ISSN: 1556-0864/08/0304-0386

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.

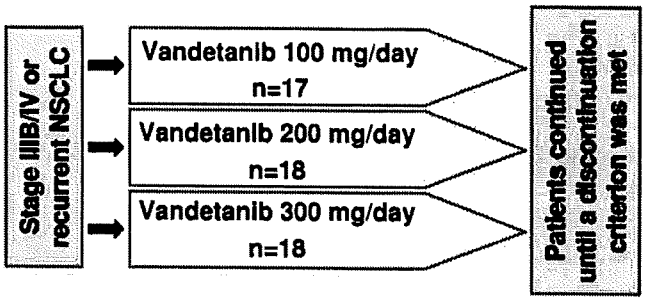

FIGURE 1. Study design.

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.

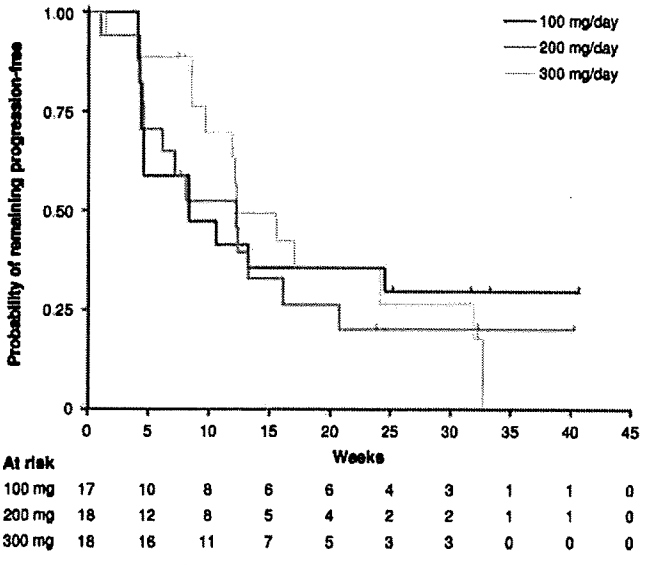

FIGURE 2. Kaplan–Meier curve for time to progression.

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.

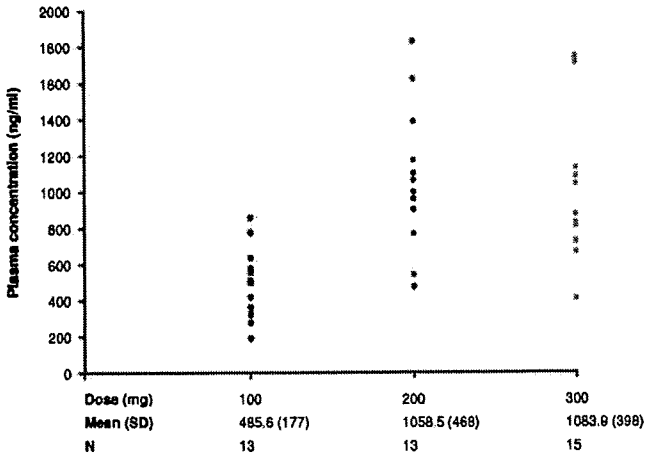

FIGURE 3. Observed maximum vandetanib plasma concentration at day 28. Patients who received dose reduction within the first 28 days were excluded.

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-

TABLE 5. Estimated Pharmacokinetic Parameters of Vandetanib[a]

	Clearance (L/h)	C_{max} (ng/ml)	Steady-state Exposure (ng/h/ml)	Half-life (d)	Accumulation Ratio
Median	10.2	1282	29,469	6.2	8.87
Minimum value	4.04	740	16,685	3.4	4.89
Maximum value	17.98	3018	74,257	13.8	19.85

[a] Simulated PK parameters if all patients (n = 51) were administered 300 mg vandetanib once a day for 56 d.

TABLE 6. Summary of Plasma Angiogenesis Biomarker Levels by Best Overall RECIST Response

Biomarker	Best Response (RECIST)	Median (range; n)		
		Baseline	Day 29	Day 57
VEGF (pg/ml)	PR	22.3 (0–264.2; n = 6)	73.2 (0–164.4; n = 6)	80.9 (28.7–183.7; n = 6)
	SD	37.0 (0–227.7; n = 16)	79.4 (38.5–281.6; n = 16)	97.4 (19.0–238.7; n = 16)
	PD	63.7 (0–897.7; n = 21)	121.0 (10.7–477.9; n = 21)	93.6 (63.9–343.2; n = 5)
	Total	51.5 (0–897.7; n = 43)	82.8 (0–477.9; n = 43)	95.5 (19.0–343.2; n = 27)
Tie-2 (ng/ml)	PR	23.5 (16.6–29.1; n = 6)	22.6 (19.8–38.8; n = 6)	23.3 (17.2–37.0; n = 6)
	SD	26.9 (6.0–33.6; n = 16)	27.4 (12.3–45.4; n = 16)	28.5 (23.3–52.4; n = 16)
	PD	28.5 (18.2–43.3; n = 21)	30.7 (18.0–56.3; n = 21)	30.2 (20.7–36.0; n = 5)
	Total	27.4 (6.0–43.3; n = 43)	29.2 (12.3–56.3; n = 43)	27.5 (17.2–52.4; n = 27)
VEGFR-2 (pg/ml)	PR	7406.5 (5564–9868; n = 6)	6418.5 (4878–8030; n = 6)	6001.5 (4846–7156; n = 6)
	SD	7577.5 (5622–8687; n = 16)	6819.5 (4666–8630; n = 16)	6450.5 (5024–8372; n = 16)
	PD	7861.0 (4981–11391; n = 21)	6910.0 (3763–11136; n = 21)	6710.0 (4131–8606; n = 5)
	Total	7721.0 (4981–11391; n = 43)	6881.0 (3763–11136; n = 43)	6563.0 (4131–8606; n = 27)

PD, progressive disease; PR, partial response; RECIST, Response Evaluation Criteria in Solid Tumors; SD, stable disease; Tie-2, soluble angiopoietin receptor; VEGF, vascular endothelial growth factor; VEGFR-2, vascular endothelial growth factor receptor-2.

tion for five patients (300 mg, n = 4; 100 mg, n = 1); none of these QTc prolongation events met the protocol criteria for QTc prolongation by subsequent central review.

No significant abnormalities in any clinical laboratory variables were observed except for an increase of alanine aminotransferase (ALT increased) with CTC grade 3 in three patients in the vandetanib 300 mg arm.

Pharmacokinetic Results

The observed vandetanib plasma concentrations at day 28 for each evaluable patient are shown in Figure 3. These data were fitted to a one-compartment model to estimate population PK parameter values for the 300 mg arm, which were found to adequately characterize the observed plasma concentrations over time, and there was a good correlation between the individual predicted and observed data (data not shown). Estimates of the half-life ranged from 3.4 to 13.8 days, with a median population value of 6.2 days. The estimated time to PK steady state was approximately 1 month. Simulations from the PK characteristics of this patient population suggest a median steady-state exposure of approximately 29,500 ng/h/ml for a 300 mg dose administered once a day and an estimated median C_{max} of 1282 ng/ml (range, 740 to 3018 ng/ml). The estimated population PK parameters for the 300 mg arm are summarized in Table 5.

Tumor Biomarkers

Twenty-seven tumor samples were available for analysis and 12 of these were evaluable for determination of *EGFR* gene copy number by fluorescence in situ hybridization. Four of 12 evaluable patients had high *EGFR* gene copy number (best overall RECIST response of SD and PD, both n = 2) whereas the remaining eight patients did not (best overall RECIST response: PR, n = 1; SD, n = 3; and PD, n = 4). Nine of 27 samples were successfully sequenced for *EGFR* exons 19–21. In addition, 21 of 27 samples had successful ARMS analysis for L858R and the most common exon 19 deletion mutation (746–750). A confirmed mutation (exon 19 deletion [746–750]) was observed in a female nonsmoker from the 200 mg arm with adenocarcinoma and a high *EGFR* gene copy number (best RECIST response of PD). Of the remaining tumor samples, 21 had no *EGFR* mutation (by DNA sequencing or ARMS analysis), and in five cases, the *EGFR* mutation status could not be determined (not evaluable by either DNA sequencing or ARMS). Tumor samples were obtained from three patients who achieved a PR. Two of these tumor samples had no *EGFR* mutation and one had an unconfirmed result by direct DNA sequencing and ARMS assay of *EGFR* exons 19–21.

Blood Biomarkers

Median plasma levels of VEGF showed a trend to increase during the study period irrespective of clinical out-

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 I 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.

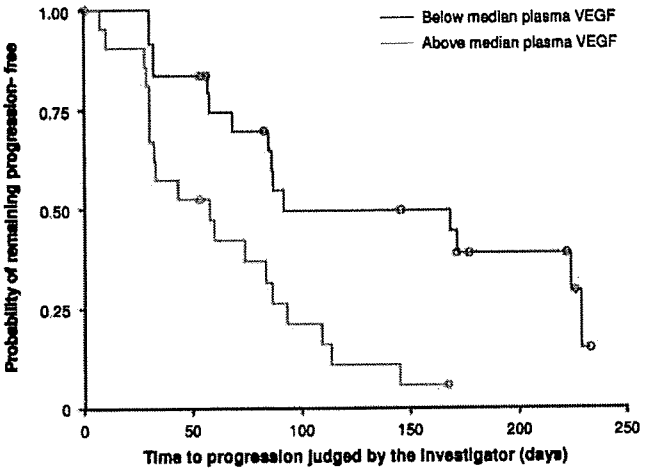

FIGURE 4. Kaplan–Meier curve of low (below median) versus high (above median) baseline plasma VEGF and time to progression.

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.

ACKNOWLEDGMENTS

This study, including editorial assistance provided by Chris Watson of Mudskipper Bioscience, was supported financially by AstraZeneca. ZACTIMA is a trademark of the AstraZeneca group of companies.

REFERENCES

1. Parkin DM, Bray F, Ferlay J, et al. Global cancer statistics, 2002. *CA Cancer J Clin* 2005;55:74–108.
2. Sandler A, Gray R, Perry MC, et al. Paclitaxel-carboplatin alone or with bevacizumab for non-small-cell lung cancer. *N Engl J Med* 2006;355: 2542–50.
3. Fukuoka M, Yano S, Giaccone G, et al. Multi-institutional randomized phase II trial of gefitinib for previously treated patients with advanced non-small-cell lung cancer. *J Clin Oncol* 2003;21:2237–46.
4. Shepherd FA, Rodrigues PJ, Ciuleanu T, et al. Erlotinib in previously treated non-small-cell lung cancer. *N Engl J Med* 2005;353:123–32.
5. Ciardiello F, Troiani T, Bianco R, et al. Interaction between the epidermal growth factor receptor (EGFR) and the vascular endothelial growth factor (VEGF) pathways: a rational approach for multi-target anticancer therapy. *Ann Oncol* 2006;17:vii109–vii114.
6. Viloria-Petit A, Crombet T, Jothy S, et al. Acquired resistance to the antitumor effect of epidermal growth factor receptor-blocking antibodies in vivo: a role for altered tumor angiogenesis. *Cancer Res* 2001;61: 5090–101.
7. Herbst RS, Johnson DH, Mininberg E, et al. Phase I/II trial evaluating the anti-vascular endothelial growth factor monoclonal antibody bevacizumab in combination with the HER-1/epidermal growth factor receptor tyrosine kinase inhibitor erlotinib for patients with recurrent non-small-cell lung cancer. *J Clin Oncol* 2005;23:2544–55.
8. Wedge SR, Ogilvie DJ, Dukes M, et al. ZD6474 inhibits vascular endothelial growth factor signaling, angiogenesis, and tumor growth following oral administration. *Cancer Res* 2002;62:4645–55.
9. Ichihara M, Murakumo Y, Takahashi M. RET and neuroendocrine tumors. *Cancer Lett* 2004;204:197–211.
10. Holden SN, Eckhardt SG, Basser R, et al. Clinical evaluation of ZD6474, an orally active inhibitor of VEGF and EGFReceptor signaling, in patients with solid, malignant tumors. *Ann Oncol* 2005;16:1391–7.
11. Tamura T, Minami H, Yamada Y, et al. A Phase I dose-escalation study of ZD6474 in Japanese patients with solid, malignant tumors. *J Thorac Oncol* 2006;1:1002–9.
12. Natale RB, Bodkin D, Govindan R, et al. ZD6474 versus gefitinib in patients with advanced NSCLC: Final results from a two-part, double-blind, randomized Phase II trial. *Proc Am Soc Clin Oncol* 2006;abst 7000.
13. Heymach J, Paz-Ares L, de Braud F, et al. Randomized phase II study of vandetanib (VAN) alone or in combination with carboplatin and paclitaxel (CP) as first-line treatment for advanced non-small cell lung cancer (NSCLC). *J Clin Oncol* 2007;25(Suppl 18):abst 7544.
14. Heymach JV, Johnson BE, Prager D, et al. Randomized, placebo-controlled phase II study of vandetanib plus docetaxel in previously treated non small-cell lung cancer. *J Clin Oncol* 2007;25:4270–7.
15. Cappuzzo F, Hirsch FR, Rossi E, et al. Epidermal growth factor receptor gene and protein and gefitinib sensitivity in non-small-cell lung cancer. *J Natl Cancer Inst* 2005;97:643–55.
16. Horiike A, Kimura H, Nishio K, et al. Detection of epidermal growth factor receptor mutation in transbronchial needle aspirates of non-small cell lung cancer. *Chest* 2007;131:1628–34.
17. Gatzemeier U, Blumenschein G, Fosella F, et al. Phase II trial of single-agent sorafenib in patients with advanced non-small cell lung carcinoma. *Proc Am Soc Clin Oncol* 2006;24:abst 7002.
18. Socinski MA, Novello S, Sanchez JM, et al. Efficacy and safety of sunitinib in previously treated, advanced non-small cell lung cancer (NSCLC): Preliminary results of a multicenter phase II trial. *Proc Am Soc Clin Oncol* 2006;24:abst 7001.
19. Thatcher N, Chang A, Parikh P, et al. Gefitinib plus best supportive care in previously treated patients with refractory advanced non-small-cell lung cancer: results from a randomised, placebo-controlled, multicentre study (Iressa Survival Evaluation in Lung Cancer). *Lancet* 2005;366: 1527–37.
20. Johnson DH, Fehrenbacher L, Novotny WF, et al. Randomized phase II trial comparing bevacizumab plus carboplatin and paclitaxel with carboplatin and paclitaxel alone in previously untreated locally advanced or metastatic non-small-cell lung cancer. *J Clin Oncol* 2004;22:2184–91.
21. Lynch TJ, Bell DW, Sordella R, et al. Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. *N Engl J Med* 2004;350:2129–39.
22. Pao W, Miller V, Zakowski M, et al. EGFReceptor gene mutations are common in lung cancers from "never smokers" and are associated with sensitivity of tumors to gefitinib and erlotinib. *Proc Natl Acad Sci USA* 2004;101:13306–11.
23. Hirsch F, Varella-Garcia M, Cappuzzo F, et al. Combination of EGFR gene copy number and protein expression predicts outcome for advanced non-small-cell lung cancer patients treated with gefitinib. *Ann Oncol* 2007;18:752–60.
24. Paez JG, Janne PA, Lee JC, et al. EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. *Science* 2004;304: 1497–500.
25. Janne PA, Engelman JA, Johnson BE. Epidermal growth factor receptor mutations in non-small-cell lung cancer: implications for treatment and tumor biology. *J Clin Oncol* 2005;23:3227–34.
26. Yoshimoto A, Kasahara K, Nishio M, et al. Changes in angiogenic growth factor levels after gefitinib treatment in non-small cell lung cancer. *Jpn J Clin Oncol* 2005;35:233–8.

Drug Metab. Pharmacokinet. 23 (2): *SNP1* (139)–*SNP9* (147) (2008).

SNP Communication

Genetic Variations and Haplotypes of *ABCC2* Encoding MRP2 in a Japanese Population

Kimie SAI[1,2,*], Yoshiro SAITO[1,2], Masaya ITODA[1], Hiromi FUKUSHIMA-UESAKA[1], Tomoko NISHIMAKI-MOGAMI[2], Shogo OZAWA[1,3,a], Keiko MAEKAWA[1,2], Kouichi KUROSE[1,4], Nahoko KANIWA[1,4], Manabu KAWAMOTO[5], Naoyuki KAMATANI[5], Kuniaki SHIRAO[6,b], Tetsuya HAMAGUCHI[6], Noboru YAMAMOTO[6], Hideo KUNITOH[6], Yuichiro OHE[6], Yasuhide YAMADA[6], Tomohide TAMURA[6], Teruhiko YOSHIDA[7], Hironobu MINAMI[8,c], Yasuhiro MATSUMURA[9], Atsushi OHTSU[10], Nagahiro SAIJO[11] and Jun-ichi SAWADA[1,2]

[1]*Project Team for Pharmacogenetics,* [2]*Division of Functional Biochemistry and Genomics,*
[3]*Division of Pharmacology,* [4]*Division of Medicinal Safety Science,*
National Institute of Health Sciences, Tokyo, Japan
[5]*Division of Genomic Medicine, Department of Advanced Biomedical Engineering and Science, Tokyo Women's Medical University, Tokyo, Japan*
[6]*Division of Internal Medicine, National Cancer Center Hospital*
[7]*Genomics Division, National Cancer Center Research Institute, National Cancer Center, Tokyo, Japan*
[8]*Division of Oncology/Hematology,*
[9]*Investigative Treatment Division, Research Center for Innovative Oncology,*
[10]*Division of GI Oncology/Digestive Endoscopy,*
[11]*Deputy Director, National Cancer Center Hospital East, Chiba, Japan*

Full text of this paper is available at http://www.jstage.jst.go.jp/browse/dmpk

Summary: The multidrug resistance-associated protein 2 (MRP2) encoded by the *ABCC2* gene is expressed in the liver, intestine and kidneys and preferentially exports organic anions or conjugates with glucuronide or glutathione. In this study, all 32 exons and the 5′-flanking region of *ABCC2* in 236 Japanese were resequenced, and 61 genetic variations including 5 novel nonsynonymous ones were detected. A total of 64 haplotypes were determined/inferred and classified into five *1 haplotype groups (*1A, *1B, *1C, *1G, and *1H) without nonsynonymous substitutions and *2 to *9 groups with nonsynonymous variations. Frequencies of the major 4 haplotype groups *1A (−1774delG), *1B (no common SNP), *1C (−24C>T and 3972C>T), and *2 [1249G>A (Val417Ile)] were 0.331, 0.292, 0.172, and 0.093, respectively. This study revealed that haplotype *1A, which has lowered activity, is quite common in Japanese, and that the frequency of *1C, another functional haplotype, was comparable to frequencies in Asians and Caucasians. In contrast, the haplotypes harboring 3972C>T but not −24C>T (*1G group), which are reportedly common in Caucasians, were minor in Japanese. Moreover, the allele 1446C>T (Thr482Thr), which has increased activity, was not detected in our Japanese population. These findings imply possible differences in MRP2-mediated drug responses between Asians and Caucasians.

Keywords: ABCC2; MRP2; genetic variation; haplotype; amino acid change

Received; October 15, 2007, Accepted; December 5, 2007
**To whom correspondence should be addressed:* Kimie SAI, Ph.D., *Division of Functional Biochemistry and Genomics, National Institute of Health Sciences, 1-18-1 Kamiyoga, Setagaya-ku, Tokyo 158-8501, Japan. Tel: +81-3-3700-9478, Fax: +81-3-3707-6950, E-mail: sai@nihs.go.jp*
[a]Present address: Department of Pharmacodynamics and Molecular Genetics, Faculty of Pharmaceutical Sciences, Iwate Medical University, Iwate, Japan.
[b]Present address: Department of Medical Oncology, OITA University Faculty of Medicine, Yufu, Japan
[c]Present address: Medical Oncology, Department of Medicine, Kobe University Hospital and Graduate School of Medicine, Kobe, Japan.
As of October 7, 2007, the novel variations reported here are not found in the database of Japanese Single Nucleotide Polymorphisms (http://snp.ims.u-tokyo.ac.jp/), dbSNP in the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/SNP/), or PharmGKB Database (http://www.pharmgkb.org/).
This study was supported in part by the Program for the Promotion of Fundamental Studies in Health Sciences of the National Institute of Biomedical Innovation, and Health and Labor Sciences Research Grants from the Ministry of Health, Labor and Welfare.

Introduction

The multidrug resistance-associated protein 2 (MRP2) or canalicular multispecific organic anion transporter (cMOAT) is a 190–200 kDa transmembrane glycoprotein comprised of 1545 amino acids and belongs to the superfamily C of ATP-binding cassette (ABC) transporters. This transporter is expressed on hepatic canalicular membranes, intestinal apical membranes, luminal membranes of renal proximal tubules, placental epithelial cells, and the blood brain barrier.[1] MRP2 exports endogenous and exogenous substances, preferentially organic anions or conjugates with glucuronide, glutathione and sulfate.[1–3] This protein originally identified in cisplatin-resistant tumor cells[4] is shown to confer drug resistance to other anti-cancer drugs, such as vincristine and doxorubicin.[5,6]

MRP2 is encoded by the *ABCC2* gene located on chromosome 10q24 and consists of 32 exons (31 coding exons) and spans 69 kb. Several *ABCC2* genetic variations have been detected in patients with Dubin-Johnson syndrome (DJS), an autosomal recessive disease characterized by hyperbilirubinemia with conjugated bilirubin or increased coproporphyrin excretion in urine.[2,7] Recent studies on *ABCC2* have identified common single nucleotide polymorphisms (SNPs) such as −24C>T and −3972C>T (Ile 1324Ile) among several ethnic populations, and several studies have suggested their association with altered MRP2 expression or function.[8–17] In more recent studies on *ABCC2* haplotypes covering an extended 5′-flanking region, close linkages were found among −1549A>G in the 5′-flanking region and two common SNPs −24C>T and −3972C>T (Ile1324Ile).[8] In addition, as possible functional SNPs, −1774delG in Koreans[8] and −1019A>G in Caucasians[10] were reported. However, there is little information on detailed haplotype structures throughout the gene, and comprehensive haplotype analysis in Japanese has not yet been conducted.

We previously analyzed *ABCC2* genetic variations within all 32 exons and the proximal 5′-flanking region (approximately 800 bp upstream of the translation initiation site) using established cell lines derived from Japanese cancer patients to obtain preliminary information on *ABCC2* SNPs in Japanese.[18] In this study, to reveal *ABCC2* haplotype structures in Japanese, we resequenced the *ABCC2* gene including the distal 5′-upstream region (approximately 1.9 kb upstream from the translation initiation site) as well as all 32 exons in 236 Japanese subjects and conducted haplotype analysis using the detected genetic polymorphisms.

Materials and Methods

Human DNA samples: Genomic DNA samples were obtained from blood leukocytes of 177 Japanese cancer patients at two National Cancer Center Hospitals (Tokyo and Chiba, Japan) and Epstein-Barr virus-transformed lymphoblastoid cells prepared from 59 healthy Japanese volunteers at the Tokyo Women's Medical University under the auspices of the Pharma SNP consortium (Tokyo, Japan). Written informed consent was obtained from all subjects. Ethical review boards of all participating organizations approved this study.

PCR conditions for DNA sequencing: We sequenced all 32 exons of the *ABCC2* gene and approximately 800 bp upstream of the translation initiation codon (proximal 5′-flanking region) as described previously and also extended the sequenced region to 1.9 kb upstream of the translation initiation site (distal 5′-flanking region). Briefly, for amplification of the proximal 5′-flanking region and 32 exons, 5 sets of multiplex PCR were performed from 200 ng of genomic DNA using 1.25 units of Z-taq (Takara Bio. Inc., Shiga, Japan) with 0.3 uM each of the mixed primers as shown in **Table 1** [1st PCR]. The first PCR conditions consisted of 30 cycles of 98°C for 5 sec, 55°C for 5 sec, and 72°C for 190 sec. Next, each exon was amplified separately using the 1st PCR product by Ex-Taq (0.625 units, Takara Bio. Inc.) with appropriate primers (0.3 uM) (**Table 1**) [2nd PCR]. The conditions for the second round PCR were 94°C for 5 min, followed by 30 cycles of 94°C for 30 sec, 55°C for 1 min, and 72°C for 2 min, and then a final extension at 72°C for 7 min. For amplification of the distal 5′-flanking region, multiplex PCR was performed from 25 ng of genomic DNA using 1 unit of Ex-Taq (Takara Bio. Inc.) with 0.4 uM each of the 2 sets of primers as shown in **Table 1** [PCR]. The PCR conditions were 94°C for 5 min, followed by 30 cycles of 94°C for 30 sec, 60°C for 1 min, and 72°C for 2 min, and then a final extension at 72°C for 7 min.

Following the PCR, products were treated with a PCR Product Pre-Sequencing Kit (USB Co., Cleveland, OH, USA) and directly sequenced on both strands using an ABI BigDye Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) with the sequencing primers listed in **Table 1** (Sequencing). Excess dye was removed by a DyeEx-96 kit (Qiagen, Hilden, Germany), and the eluates were analyzed on an ABI Prism 3700 DNA Analyzer (Applied Biosystems). All variations were confirmed by sequencing PCR products generated from new amplifications from genomic DNA. Genbank NT_030059.12 was used as the reference sequence.

Linkage disequilibrium (LD) and haplotype analyses: Hardy-Weinberg equilibrium and LD analyses were performed using SNPAlyze 3.1 software (Dynacom Co., Yokohama, Japan). Pairwise LDs were shown as rho square (r^2) and $|D'|$ values in **Figure 1**. Diplotype configurations (haplotype combinations) were inferred by LDSUPPORT software, which determined the posterior probability distribution of diplotype configurations for each subject based on estimated haplotype frequencies[19].

Results and Discussion

In this study, sixty-one *ABCC2* genetic variations including 36 novel ones were detected in 236 Japanese subjects

Table 1. Primer sequences used in this study

Amplified or sequenced region	Forward primer (5′ to 3′)	Reverse primer (5′ to 3′)	Amplified region[a]
PCR (Ex-taq)			
5′-Flanking (for −1.9 k to −1.7 k)	CCACCAGTGCCAAGAGAAGTAT	CACAAGTCATCTGGAAAACACA	20289134–20289443
5′-Flanking (for −1.7 k to −950)	ATGAGGTGGTATCTAACTGTGG	AAATGTTTTCTGTAGGGACGGG	20289392–20290182
1st PCR (Z-taq)			
5′-Flanking (for −1.2 k) to exon 6	ATACTGCATGGGTGGTTATG	AACCTGCCTCCAAATTTTTC	20289942–20303347
Exons 7 to 11	GGAGAATCACTTTGAAGCCG	CTAGCAAGTGTGAGGGGTGT	20304874–20314079
Exons 12 to 19	TCTGTGAATGTGGCAAAACT	GGATCTACCAAGAATTTAGC	20315189–20328004
Exons 20 to 25	GATGAGCATTTTCAATTTAC	TCAGTTCACCCAGCACTTAT	20338211–20344941
Exons 26 to 32	GAGCAAGACCTTGTCTCATA	CCATGGATGAATCTCAGATA	20349821–20360334
2nd PCR (Ex-taq)			
5′-Flanking (for −880 to −130)	GGAAGATCGCTTGAACCCAT	TCATCCCAACCATTTAATCG	20290245–20290994
Exon 1	TTGTTGGCCAGCTCTGTTG	TTCTGGTTCTTGTTGGTGAC	20290810–20291254
Exon 2	GGGTAAGGCTGGATATGGAT	CTGGCTCTACCTGAGACAAT	20292767–20293194
Exon 3	CACCGGAAACCATTCTGTTC	TTTGCCTCACTATGGATCCC	20300442–20300773
Exon 4	GCCAGATTAGTCACGACAGT	CCAAAGGAAGTCTACATGGCC	20301708–20302134
Exon 5	CAGGTAAGGAAAAAAAGAGTGG	CCTTGTCATAAAATGGTCTG	20301966–20302418
Exon 6	TATGCCAGAAAATCTGATTA	AGGTGGAACATGAGCTTGAGT	20302499–20303070
Exon 7	GGTGGAGATAGCCTCTGACC	TGCACTGAGAAGTATGAAGTGC	20305320–20305728
Exon 8	CCTGTACAGAGAAGGCCACG	TGCGGTCTTCATGAACACAA	20307385–20307816
Exon 9	GGCTTTGGACAATTCTGGTC	TCCACCCATTGTCTGTGAAC	20308539–20309038
Exon 10	AGGCAAGAAGTCACAGTGCC	TTGCCCAAACTCCCATTAAG	20312158–20312650
Exon 11	ACAGTCAGGCAAGGGCTATG	GACAGGAGGACATGAAACAA	20313420–20313873
Exon 12	GATTTCTATTCCCCACATTT	GAGCTGGGGGTATGGTACAA	20315554–20315983
Exon 13	GTGACCTTGGAGAAGATATT	CTCTTGAAAGTTTACCAGCA	20316189–20316623
Exon 14	TTGCTCAAGGACTGAAATAG	CCTGCTTATCCTCAGAAGAG	20318223–20318732
Exon 15	GGTCTCATGGTCTCATTCTA	GGGTTTATCCTGCACTAGTA	20319650–20320025
Exon 16	AGAAGCACTTTGGGGTCTTGTA	GCTGAAATGGGAAGGAGAATC	20321144–20321581
Exon 17	GCTGAAAAACGATAGTCCAA	TCAACTAGATTACCCCTGTGT	20325354–20325863
Exons 18 and 19	TCACAGGGTGACAAGCAAC	TTGAATCTCTGGGTAGTTTG	20326820–20327678
Exon 20	GAAACCAGCAAGATCAGAGGA	TCACTCAGCTGGCATCAAAG	20338493–20338929
Exon 21	TGACTGTGACATCTGCTTGC	GGACAGAGGACATATTGCTCC	20338927–20339248
Exons 22 and 23	GCATTGTATTTCAGCATTGT	ACAGTGTTGTCTAGGGGGAC	20339701–20340506
Exon 24	GAACACACAGAATCCAACAGA	TCACTTCAGCTTCAGACAGT	20342562–20343001
Exon 25	TCTCATTGGTCTCCTCCTCG	AATTTCACACCACTAGCCAT	20344186–20344672
Exon 26	GAGGCATTGCCTAAGAGTGC	AAAGATGGAGCCAGGGTTTG	20350122–20350523
Exons 27 and 28	GGCAAGGATTGTCTTTCTTA	CGACAGCTGCGGTAAGTCTG	20351928–20352954
Exon 29	AGAGATGGAGTAGCCAGTCAC	CAGCCACAAATGCATATTACC	20353790–20354262
Exon 30	GAAGCTCAACCACAAACCAG	GCTCGACCAGTTTTCAAGAG	20355106–20355610
Exon 31	GCAAGGTACAGCTAGTTGAA	GCGTGATGTAAAATTTTGGC	20358730–20359248
Exon 32	GCTGTGGCTCATTGATTTTC	AAGGTGATAAAACAGAAATG	20359651–20360213
Sequencing			
5′-Flanking (for −1.7 k)	CCACCAGTGCCAAGAGAAGTAT	CACAAGTCATCTGGAAAACACA[b]	
(for −1.7 k to −1.3 k)	GGTATCTAACTGTGGTTTTG	GAAGGAAAGGAGTCAAAGGAAC	
(for −1.5 k to −950)	TCCCACACTGAATGCTGCCTTT	TAGGGACGGGGTCTCACTAT	
(for −880 to −400)	GGAAGATCGCTTGAACCCAT[b]	ATGTGCAGTTTCGCTTCTG	
(for −570 to −130)	CATATAGGCTCACACTGGAT	TCATCCCAACCATTTAATCG[b]	
Exon 1	TGGTTCCTTTTATGTATGGC	GTTCTTGTTGGTGACCACCC	
Exon 2	AAAGCAGTGGGATGTGCTG	TGTCTCTACTGTGCACCAAGG	
Exon 3	CACCGGAAACCATTCTGTTC[b]	TTTGCCTCACTATGGATCCC[b]	
Exon 4	CCTCCTTTCTTCCCATGTTC	CTCAACTTGATGCCATTTAC	
Exon 5	TGGGGCAACCTCTAACTCATA	TGAGACCCAGACATCTTAAA	
Exon 6	TTAGGGTCTCCAAATAAACA	ACTTTCAGAGGAGTGAGAGAGT	
Exon 7	GGTGGAGATAGCCTCTGACC[b]	TGCACTGAGAAGTATGAAGTGC[b]	
Exon 8	CCTGTACAGAGAAGGCCACG[b]	CACAATGCTGTAAGGTTAAG	
Exon 9	GGCTTTGGACAATTCTGGTC[b]	TCCACCCATTGTCTGTGAAC[b]	
Exon 10	GTGCCTTGGAGAAGCTGTGT	TTGCCCAAACTCCCATTAAG[b]	
Exon 11	TCACTGGGCACCTCAAGTTC	GGAATCCATCACCTCTACCA	
Exon 12	ACATTTTGGGGACTATATCT	ATGCCAGCTAGTCTATCAAA	
Exon 13	GGAGGCTGGATGATCCTTAAG	CTCTTGAAAGTTTACCAGCA[b]	
Exon 14	CATCTGTCTATGGTGGGATA	ATAGGCTCAAGACAAATCTC	
Exon 15	GATTTCATTCACCTCCTGTT	CATTTCCCCATGCATTCTAT	
Exon 16	CCAATCTTGAGGGGAAATCT	TCCAAGACCTCACCTACTAGC	