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

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

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

PATIENTS AND METHODS

Study Design

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

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

Patient Eligibility

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

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

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

Treatment

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

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

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

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

Safety Assessments

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

Pharmacokinetic Analysis

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

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

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

Efficacy Assessments

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

RESULTS

Patients

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

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

Safety

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

Table 1. Baseline characteristics of patients

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

[a]Eastern Cooperative Oncology Group performance status.

Table 2. No. of patients with drug-related adverse events that occurred in ≥20% of patients receiving lapatinib

	Dose (mg/day)[a]												No. of patients (%)
	900			1200			1600			1800			
Common terminology criteria grade	1	2	3	1	2	3	1	2	3	1	2	3	
Any adverse events	3	3	0	4	2	0	1	4	1	2	2	2	24 (100)
Gastrointestinal	1	1	0	4	0	0	2	3	1	3	1	2	18 (75)
Diarrhea	1	1	0	4	0	0	2	1	1	3	1	2	16 (67)
Stomatitis	0	0	0	1	0	0	1	2	0	1	0	0	5 (21)
Skin	4	2	0	3	1	0	4	2	0	4	2	0	22 (92)
Rash	1	0	0	4	0	0	1	2	0	3	2	0	13 (54)
Dry skin	5	0	0	2	0	0	1	0	0	0	0	0	8 (33)
Seborrheic dermatitis	3	1	0	0	0	0	0	0	0	1	0	0	5 (21)
Paronychia	0	1	0	0	1	0	2	0	0	1	0	0	5 (21)
Metabolism and nutrition	1	0	0	1	0	0	2	0	0	4	0	0	8 (33)
Anorexia	0	0	0	1	0	0	1	0	0	3	0	0	5 (21)
Investigations	2	1	0	3	2	0	3	1	0	3	1	1	17 (71)
Decreased lymphocyte count	0	1	0	1	1	0	0	1	0	1	0	0	5 (21)

[a]Six patients at each dose level.

patients at each dose level. The majority of events was mild (Grade 1–2); the most common events were skin reactions (mostly rash and dry skin) observed in 22 patients (92%) and gastrointestinal disorders (mostly diarrhea) in 18 patients (75%). The most severe drug-related adverse events were Grade 3 diarrhea observed in one patient at 1600 mg dose level and two patients at 1800 mg dose level. One of these also had Grade 3 γ-GTP increase. All diarrhea resolved with routine symptomatic treatment during or after withdrawal of lapatinib therapy, γ-GTP increase resolved without further treatment after completion of lapatinib therapy.

Grade 1/2 drug-related nausea and vomiting were experienced only by patients at higher dose levels of lapatinib [1/6 (17%) at 1600 mg/day and 3/6 (50%) at 1800 mg/day], with Grade 2 symptoms only seen at the 1800 mg dose level.

For other adverse events, no clear drug relation was found. The most frequent events included decreased body weight and serum alkaline phosphatase increase, each observed in 10 patients (42%). Grade 1 drug-related decreases in left ventricular ejection fraction were found in three of the six patients at the 1200 mg dose level. No clinically relevant changes in vital signs, 12-lead electrocardiogram or echocardiography were noted.

Hypoxemia and pneumonia were reported at the 900-mg dose level in another patient with NSCLC on Day 35. After hypoxemia occurred, the patient continued to receive study drug medication until Day 40. We attributed hypoxemia to bronchostenosis caused by the primary disease. Oxygen inhalation and erythromycin were given and hypoxemia improved while the pneumonia was resolved on Day 41 before the patient died from progression of primary disease 3 months after the events were resolved. Chest X-rays and CT findings for this patient were inconsistent with those for interstitial pneumonia associated with other tyrosine kinase inhibitors; therefore a drug relation with lapatinib was denied.

Maximum Tolerated Dose

Dose escalation was stopped at 1800 mg/day, where two patients experienced DLT (Grade 3 diarrhea). One of these patients also experienced Grade 3 γ-GTP increase. Thus, 1800 mg/day was determined as the MTD.

Pharmacokinetics

Table 3 shows the PK parameters derived from data on 23 patients (data from one patient received lapatinib for only 19 days and are not included).

Serum concentrations of lapatinib at each dose level on Days 1 and 21 are shown in Fig. 1. Repeated doses of lapatinib (900–1800 mg/day) for 21 days resulted in dose-related increases in mean C_{max} (range, 1715–3111 ng/ml) and mean AUC_{0-24} (range, 25 680–51 099 ng·h/ml) (Table 3). Large inter-patient variations were found in mean C_{max} and mean AUC_{0-24}. After a single dose of lapatinib, t_{max} was ~4 h, although values varied greatly among patients. After 21 days of treatment, t_{max} values were similar to those observed after the single dosing on Day 1.

Table 3. Derived pharmacokinetic parameters of lapatinib (including 95% confidence intervals)

Dose (mg/day)[a]	Geometric mean C_{max} (ng/ml)		Mean CSS_{max} (ng/ml)	Median t_{max} (h)		Geometric mean AUC (h ng/ml)[b]		Median $t_{1/2}$ (h)	
	Day 1	Day 21	Day 21	Day 1	Day 21	Day 1	Day 21	Day 1	Day 21
900	1011 (694–1472)	1895 (1319–2721)	857 (386–1234)	4.0 (2.0–6.0)	4.0 (3.0–6.0)	17 577 (11 812–26 154)	29 272 (21 618–39 638)	12.9 (10.1–18.3)	23.1 (9.8–38.2)
1200	1027 (474–2227)	1715 (965–3048)	820 (226–1308)	3.5 (2.1–6.0)	3.6 (3.0–7.9)	15 441 (7410–32 176)	25 680 (13 728–48 038)	11.5 (10.1–19.5)	16.9 (15.1–34.3)
1600	1538 (1042–2268)	3111 (1937–4996)	1899 (818–4357)	4.0 (2.0–8.0)	5.1 (0.9–8.0)	26 361 (17 519–39 665)	51 099 (28 674–91 062)	13.9 (9.6–18.0)	26.2 (12.9–48.3)
1800	1227 (465–3242)	2333 (927–5870)	1528 (586–3393)	3.9 (3.0–8.0)	3.9 (3.0–7.3)	32 841 (18 884–57 114)	39 451 (14 909–104 391)	15.7 (11.0–133.1)	21.8 (18.5–104.5)

AUC, area under the plasma drug concentration–time curve; C_{max}, maximum serum concentration; CSS_{max}, mean steady state maximum serum concentration; t_{max}, time to reach C_{max}; $t_{1/2}$, terminal half-life.
[a]Six patients at 900, 1200 and 1600 mg/day and five at 1800 mg/day.
[b]Day 1, AUC from 0 to infinity; Day 21, AUC from 0 to 24 h.

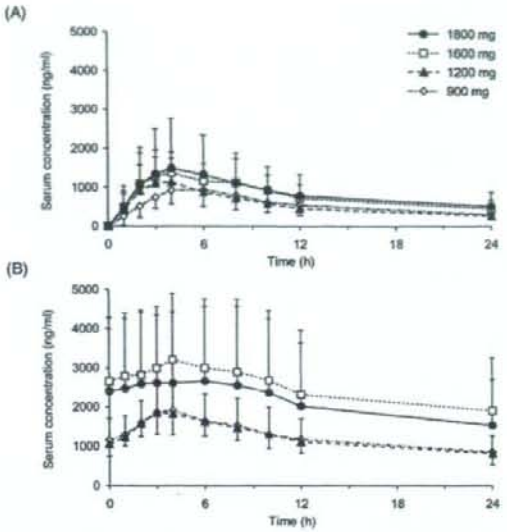

Figure 1. Serum concentrations of lapatinib at each dose level as detected on (A) Day 1 and (B) Day 21.

Steady-state serum concentrations of lapatinib generally increased with dose, 820 ± 448 ng/ml at 1200 mg dose level and 1899 ± 1356 ng/ml at 1600 mg dose level (Table 3). Both concentrations exceeded the half maximal inhibitory concentration values for *in vitro* tumor growth (14). The median $t_{1/2}$ after repeat dose was 16.9 h (range, 15.1–34.3) at 1200 mg dose level and 26.2 h (range, 12.9–48.3) at 1600 mg dose level.

The fraction of urinary excretion of lapatinib was <0.1% of the dose, suggesting that none or negligible amount of drug is excreted in urine.

Comparison of on-treatment C_{max} and AUC_{0-24} values obtained in Japanese and western patients are shown in Fig. 2 (43,44).

Efficacy

Among 24 patients, the best overall response was assessed as partial response (PR) in two patients (8.3%), stable disease (SD) in 12 patients (50.0%), progressive disease in eight patients (33.3%) and indeterminate in two patients (8.3%).

Of the two patients with PR, the first was a 73-year-old man with NSCLC (squamous cell carcinoma) with prior docetaxel and gemcitabine treatment, who received lapatinib 900 mg/day. PR was assessed by CT scan with 41% shrinkage on Day 49. Time to progression was 191 days. The second patient was a 55-year-old woman with trastuzumab-resistant breast cancer (invasive ductal carcinoma; hormone receptor-negative, ErbB-2 3+). Disease progressed after doxorubicin and cyclophosphamide/docetaxel therapy, was

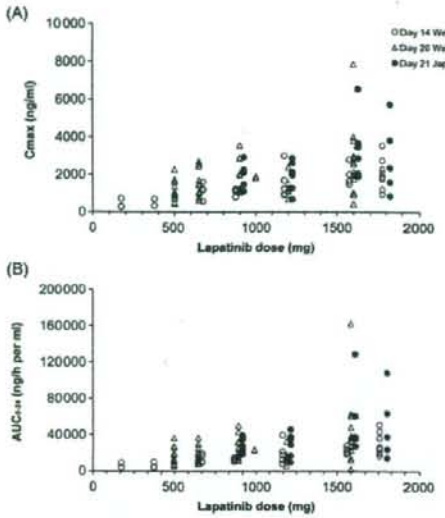

Figure 2. Relation between dose of lapatinib and exposure: comparison of (A) maximum serum concentration (C_{max}) and (B) area under the plasma drug concentration–time curve from 0 to 24 h (AUC_{0-24}) after dosing on Day 21 (our study, Japanese patients) and Days 14 and 20 (US studies, western patients).

stable with doxifluridine, and progressed with trastuzumab. Following treatment with lapatinib 1600 mg/day, the tumor shrank by 41% on Day 21. Time to progression was 133 days.

Among the patients with SD, three (two with NSCLC and one with colorectal cancer) were stabilized for >6 months and three (two with NSCLC and one with cervical cancer) were stabilized for 3–6 months and therefore were considered as having a durable response.

DISCUSSION

The dual ErbB-1/-2 inhibitor lapatinib taken orally once daily for ≥21 days was well tolerated at doses of 900–1600 mg in Japanese solid tumor patients. Adverse events were mostly mild in nature, and only four grade ≥3 drug-related adverse events were noted, in three patients (three events of Grade 3 diarrhea and one Grade 3 γ-GTP increase). No NCI-CTC Grade 4 adverse events were observed. Grade 1–2 diarrhea occurred in some patients other than those who experienced Grade 3 diarrhea; for these, supportive therapy was given and fully recovered in all cases. Grade 1/2 drug-related nausea and vomiting were experienced only by patients at higher dose levels of lapatinib, with Grade 2 symptoms only seen at 1800 mg dose level.

The types and incidences of drug-related adverse events in Japanese patients were similar to those reported from studies conducted in healthy volunteers (18) and two overseas Phase I studies, the latter including a parallel study in western patients that used similar dose administration and dose-escalation schedules (43,44). In that study as well as in ours, diarrhea and rash were the most frequently noted drug-related adverse events. Adverse events were generally mild (Grade 1–2), transient and reversible on dose delay or interruption. Headache, which was common in western patients (18), was reported only by one patient at 1600 mg dose level. 1800 mg/day was considered as MTD, at which Grade 3 diarrhea and γ-GTP increase were observed.

Skin-related adverse events of lapatinib were similar to those reported for other agents that target ErbB-1; rash is also a common adverse event associated with the ErbB-1 tyrosine kinase inhibitors gefitinib (46–49) and erlotinib (7,50), as well as the anti-ErbB-1 antibody cetuximab (51). Patients who received these medications also experienced diarrhea (7,46–50). These adverse events occurred at a similar frequency in our study as in two overseas Phase I studies (43,44).

Apart from one event of γ-GTP increase, no Grade ≥3 abnormal laboratory test suggestive of liver dysfunction was noted. Therefore, drug-related liver abnormality was generally less frequently seen with lapatinib compared with gefitinib (48,49).

Hematologic toxicity was uncommon and limited to cases of anemia. This finding is similar to those of the Phase I biomarker study (44) and studies of gefitinib (48,49,52).

None of the patients developed interstitial lung disease, which is an adverse event reportedly associated with gefitinib (53,54) and occurs in 5.8% of Japanese patients (55). However, because of the limited number of patients in our study, further studies are required to assess safety of lapatinib in this regard.

Cardiotoxicity is a known adverse event associated with trastuzumab therapy and might be related to ErbB-2 inhibition (2,56); however, we found no evidence of drug-related cardiac dysfunction in our study.

PK parameters such as C_{max} and AUC_{0-24} in this study were analyzed and their means and 95% confidence intervals compared with those obtained at similar doses (900–1800 mg) in two overseas Phase I studies (43,44). As can be seen in Fig. 2, the values were comparable among the three studies. However, large inter-patient variations were noted, especially in Japanese patients, and these might have contributed to higher mean values. On the other hand, no clear pharmacokinetic differences were apparent between Japanese and non-Japanese subjects, suggesting that values obtained overseas can be extrapolated to the Japanese population.

The dose recommended for further clinical studies outside Japan, 1500 mg/day, can be used for Phase II studies in Japan. We base this recommendation on the similar PK profiles of lapatinib in Japanese and western patients, evidence of antitumor activity at doses of ≥900 mg/day, and an MTD of 1800 mg/day.

To conclude, lapatinib, taken continuously as once-daily oral therapy at 900–1600 mg, was well tolerated in Japanese

patients with solid tumors. The safety and PK profiles shown in this study are similar to those in Phase I studies conducted in western patients. Phase II studies to determine the efficacy of lapatinib against a range of tumors are now in progress.

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Conflict of interest statement

The author, Hironobu Minami, receives honoraria from GlaxoSmithKline. The authors, Masayuki Kanezaki, Akihira Mukaiyama, and Yoshiyuki Minamide are employed by GlaxoSmithKline.

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

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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

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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.

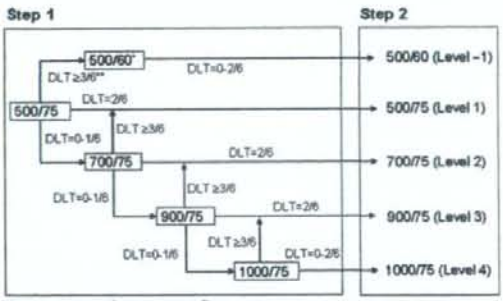

Figure 1. Scheme of dose-escalation Steps 1 and 2. DLT, dose-limiting toxicity.

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%),

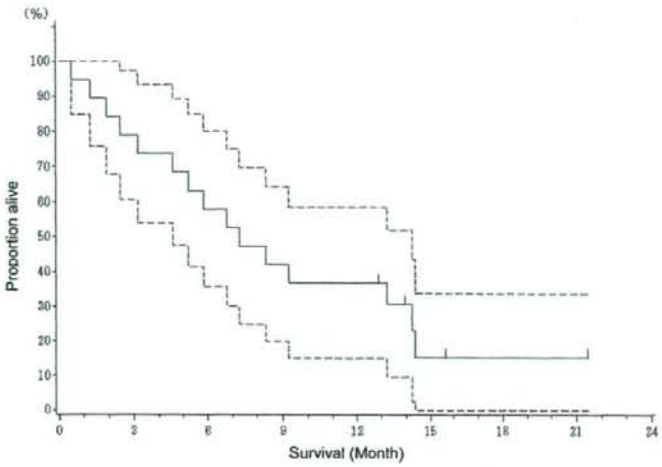

Figure 2. Kaplan–Meier plot of overall survival in the efficacy analysis set. Solid lines, overall survival; dotted lines, high and low limits of 95% confidence interval.

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

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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.

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ORIGINAL PAPER

EGFR R497K polymorphism is a favorable prognostic factor for advanced lung cancer

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Abstract
Introduction It has been reported that the R497K polymorphism of the epidermal growth factor receptor (*EGFR*) gene has attenuated functions in ligand binding, tyrosine kinase activation, and growth stimulation. On other hand, *EGFR* gene mutations at kinase domain in non-small cell lung cancer (NSCLC) have been examined for their ability to predict sensitivity to gefitinib or erlotinib.
Materials and methods We investigated the *EGFR* mutations and/or R497K polymorphism statuses in 225 surgically treated NSCLC cases. 192 adenocarcinoma cases were included. The presence or absence of *EGFR* polymorphism of exon 13 was analyzed by PCR–RFLP method.
Results *EGFR* mutations at kinase domain were found from 95 of 225 lung cancer patients. In 86.2% of patients, homo- or heterozygous Lys497 allele was present. No correlation existed between R497K *EGFR* genotype and clinico-pathological features, such as gender, smoking status, and pathological subtypes.
Conclusions *EGFR* mutation status was not correlated with R497K*EGFR* genotype of lung cancers. In node-negative patients, R497K*EGFR* genotype was not correlated with disease outcome. In node-positive patients, however, R497K *EGFR* was significantly associated with better overall survival. This association was attributable to neo-adjuvant or adjuvant chemotherapy. In 46 total gefitinib treated NSCLC patients, the prognosis was not different between the *EGFR* wild type (GG) patients and AG+AA patients. R497K*EGFR* polymorphism might be associated with favorable prognosis of advanced lung cancers and correlated with chemosensitivity.

Keywords EGFR · Lung cancer · Polymorphism · R497K

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Introduction

Lung cancer is a major cause of death from malignant diseases, due to its high incidence, malignant behavior, and lack of major advancements in treatment strategy (Ginsberg et al. 1993). There are much accumulated evidences that epidermal growth factor receptor (*EGFR*) and its family member are strongly implicated in the development and progression of numerous human tumors, including lung cancer (Nicolson et al. 2001; Onn et al. 2004). The *EGFR* tyrosine kinase inhibitor, gefitinib, was approved in Japan for the treatment of non-small cell lung cancer (NSCLC) since 2002. In 2004, two reports have shown that *EGFR* mutation statuses at tyrosine kinase (TK) domain in NSCLC patients were correlated with the clinico-pathological features related to good response to gefitinib (Paez et al. 2004; Lynch et al. 2004). *EGFR* mutations in lung cancer have been correlated with clinical response to gefitinib therapy in vivo and in vitro (Paez et al. 2004; Lynch et al. 2004; Pao et al. 2004). Genomic

profiling of the EGFR signaling is also helpful in identifying lung cancer patients who are at risk of tumor recurrence and those who are more likely to benefit from chemoradiation therapy. For example, the NSCLC patients with more than 35 (CA)n repeats in *EGFR* intron 1 polymorphism had a significantly longer overall survival than the patients with the 35 or fewer (CA)n alleles, who received radiation (RT; 50.4 Gy) or RT concurrent with chemotherapy (CT; four cycles of cisplatin plus etoposide) (Dubey et al. 2006; Keller et al. 2000). *EGFR* intron 1 and −216G/T polymorphisms influenced clinical outcomes in gefitinib-treated NSCLC patients (Liu et al. 2008). A polymorphic variant *EGFR* arising from a single nucleotide change (G–A) leading to an arginine (Arg) to lysine (Lys) substitution in codon 497 (R497K) in the extracellular domain of EGFR has been identified (Moriai et al. 1994). This polymorphism alone or in combination with another polymorphism in the same gene is associated with a lower recurrence of tumor in rectal cancer patients treated with chemoradiation (Zhang et al. 2005). To determine this *EGFR* polymorphism status and correlation with clinicopathological features in Japanese lung carcinoma, we investigated *EGFR* gene status by PCR–RELP method and direct sequencings. The findings were compared to the clinicopathologic features of lung cancer.

Materials and methods

Patients and samples

The study group included 206 lung cancer patients who had undergone surgery at the Department of Surgery II, Nagoya City University Medical School between 1997 and 2005. Fifty eight patients were treated with platinum-based neoadjuvant or adjuvant chemotherapy. Twenty seven patients were treated with gefitinib for their recurrence of lung cancer after they had undergone surgery. We have also investigated *EGFR* R497K status for 19 NSCLC patients who had treated with gefitinib for their recurrence of lung cancer after undergone surgery at the National Hospital Organization, Kinki-chuo Chest Medical Center. The lung tumors were classified according to the general rule for clinical and pathological record of lung cancer in Japan, as well as WHO classification. All tumor samples were immediately frozen and stored at −80°C until assayed.

The clinical and pathological characteristics of the 225 lung cancer patients were as follows; 132 (58.6%) were male and 93 were female. One hundred and ninety two were diagnosed as adenocarcinoma, and 33 were diagnosed as other types of carcinoma (20 squamous cell carcinomas, eight adenosquamous carcinomas and five large cell carcinomas). One hundred and twenty five (55.6%) were smoker (current smoker or ever smoker) and 100 were non-smoker. Written informed consent was obtained from the patients, and the institutional ethics committee of the Nagoya City University approved the study.

PCR assays for *EGFR* polymorphism

Genomic DNA was extracted using Wizard SV Genomic DNA purification Systems (Promega) according to the manufacturers' instructions. *EGFR* mutation statuses at kinase domain were investigated using TaqMan PCR assay (Applied Biosystems). The sequences of 13 allele-specific TaqMan MGB probes and primer sets used in the TaqMan PCR assay were already shown (Endo et al. 2005). The results of TaqMan PCR assays were already reported. The R497K *EGFR* (G–A) polymorphism was examined by PCR–RELP method as described previously (Zhang et al. 2005; Wang et al. 2007). Briefly, the PCR reactions were performed using LA-Taq kit (Takara Bio Inc, Shiga, Japan) in a 50 μl reaction volume. The primer sequences for *EGFR* gene at exon 13 were as follows: the forward primer, 5′-TGCTGTGACCCACTCTGTCT-3′ and the reverse primer, 5′-CCAGAAGGTTGCACTTGTCC-3′. The cycling conditions were as follows: initial denaturation at 95°C for 3 min, followed by 35 cycles at 94°C for 60 s, 59°C for 60 s, 72°C for 60 s. The products were purified by Qiagen PCR purification kit (Qiagen, Valencia, CA), and then digested by BstN1 restriction enzyme (New England Biolabs) at 60°C for 16 h. These samples were separated on 4% ethidium bromide-stained agarose gels. In some cases, direct sequencing were performed and analyzed by BLAST and chromatograms by manual review.

Statistical analysis

Statistical analyses were done using the Mann–Whitney *U* test for unpaired samples and Wilcoxon's singed rank test for paired samples. Linear relationships between variables were determined by means of simple linear regression. Correlation coefficients were determined by rank correlation using Spearman's test and χ^2 test. The overall survival of lung cancer patients was examined by the Kaplan–Meier methods, and differences were examined by the Log-rank test. All analysis was done using the Stat-View software package (Abacus Concepts Inc. Berkeley, CA), and was considered significant when the p value was less than 0.05.

Results

EGFR gene mutation status

Of 225 patients, in exon 19, 51 patients had the deletion type mutation. In exon 18 or exon 21, 39 patients had the

missense point mutations (1 G719S, 3 G719C, 34 L858R and 1 L861Q). Five patients had exon 20 insertion mutations (Sasaki et al. 2007). Of these 95 patients, 34 were male and 61 were female. Sixty seven were non-smokers and 28 were smokers. Ninety two patients had adenocarcinoma and three had adenosquamous cell carcinoma. Thus *EGFR* mutation statuses at exon 18–21 were significantly correlated with gender ($p < 0.0001$), tobacco-smoking ($p < 0.0001$), and pathological subtypes (adenocarcinoma vs. non-adenocarcinoma, $p < 0.0001$). Of 206 patients from Nagoya City University, 97 (51.5%) were stage I. There was a higher *EGFR* mutation in stage I (51/97, 28.4%) than in stage II–IV (33/89, 19.7%, $p = 0.0235$).

EGFR polymorphism at exon 13

Using the PCR–RFLP assay, a sequence difference in exon 13 (R497K) was found in tumors that defined in the *EGFR* gene. Example of the *EGFR* gene analyzed by PCR–RELP method was shown in Fig. 1. Same codon 497 polymorphism of *EGFR* was found in both DNAs isolated from several lung cancer samples and adjacent peripheral blood samples. Several samples were also confirmed by direct sequencing (Fig. 2). Of 225 patients, 194 patients had the *EGFR* polymorphism (80 AA and 114 GA), 117 were male and 77 were female, 110 were non-smokers and 84 were smoker, and 166 patients had adenocarcinoma and 28 had other types of lung cancers. The R497K polymorphism did not correlate with gender ($p = 0.2410$), smoking status

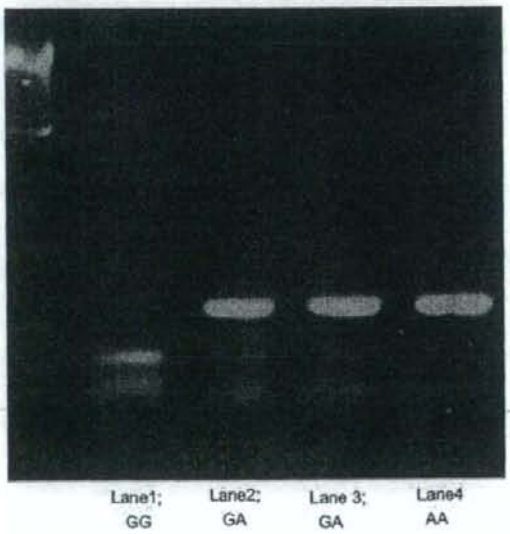

Fig. 1 Representative PCR–RELP patterns of different EGFR codon 497 status. PCR products after being digested by BstN1 were separated by agarose gel electrophoresis

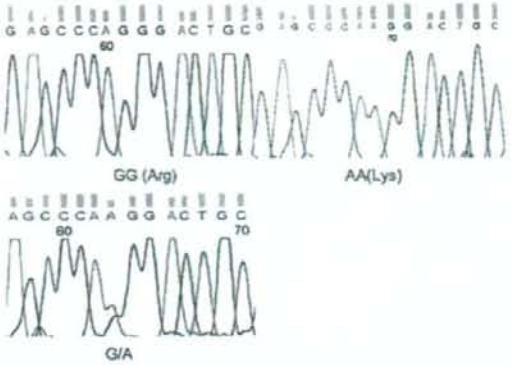

Fig. 2 The sequence results of *EGFR* exon 13. *Left upper* wild type (GG). *Right upper* heterozygous change (GA). *Left lower* homozygous change (AA)

Table 1 Clinico-pathological data of 225 lung cancer patients

Factors	EGFR		*p*-value
	GG	GA+AA	
	Patients	Patients	
Mean age (years) 63.2 ± 10.3	63.4 ± 10.0	62.0 ± 12.0	0.6685
Gender			
Male	15 (48.4%)	117 (60.3%)	0.2410
Female	16 (51.6%)	77 (49.7%)	
Smoking			
Non-smoker	16 (51.6%)	84 (43.3%)	0.4387
Smoker	15 (48.4%)	110 (56.8%)	
Pathological subtype			
Adeno	26 (83.9%)	166 (85.6%)	0.7865
Others	5 (16.1%)	28 (14.4%)	
EGFR mutation			
Positive	14 (45.2%)	81 (41.8%)	0.5566
Negative	17 (54.8%)	113 (58.2%)	
Age			
≤60	12 (38.7%)	72 (39.1%)	>0.9999
>60	19 (61.3%)	112 (60.8%)	
Pathological stages			
I	10 (35.7%)	96 (53.9%)	0.1073
II	4 (14.3%)	29 (16.3%)	
III–IV	14 (50.0%)	53 (29.8%)	
Lymph node metastasis			
Negative	14 (50.0%)	118 (66.3%)	0.1366
Positive	14 (50.0%)	60 (33.7%)	

**EGFR* epidermal growth factor receptor, *Smoker* current smoker or ever smoker, *Adeno* adenocarcinoma

($p = 0.4387$), pathological subtypes ($p = 0.7865$), and *EGFR*-TK mutation status of lung cancer ($p = 0.5566$) (Table 1). Major components of adenocarcinomas with

R497K were as follows; acinar 58.3%, solid 25.0%, and papillary 12.5%. Major components of adenocarcinomas with wild type (Lys/Lys) were as follows; acinar 40.0%, papillary 40.0%, and solid 20.0%. Thus polymorphism status did not correlated with the major components of adenocarcinomas. No significant association between R497K *EGFR* genotype and patient outcome was seen for the 206 patients from Nagoya City University ($p = 0.1121$). Pathological stages ($p < 0.0001$) but not gender ($p = 0.0696$) was a prognostic factor. In node-negative patients, 119 (28 were dead) were R497 K*EGFR* and 14 (three were dead) were wild type *EGFR*. Thus *EGFR* genotype was not correlated with disease outcome (Log-rank test $p = 0.8882$) (Fig. 3). In node-positive patients, however, 59 (33 were dead) were R497K *EGFR* and 14 (12 were dead) were wild type. Thus R497K *EGFR* was significantly associated with better overall survival (Log-rank test, $p = 0.0072$) (Fig. 4). In this cohort, pathological stage (stage II, $n = 17$ vs. stage III–IV, $n = 56$, $p = 0.2932$) or gender (male, $n = 41$ vs. female, $n = 32$, $p = 0.7957$) was not a prognostic factor. Multi-variate analysis showed that R497K status was a prognostic factor ($p = 0.0104$, relative risk 2.4, 1.229–4.689). We also compared associations between *EGFR* polymorphism status and patient outcome who were treated with platinum-based adjuvant or neo-adjuvant chemotherapy who had undergone surgery. The overall survival of 58 lung cancer patients with follow-up through March 1, 2008 was studied in reference to the *EGFR* polymorphism status. Ten were wild type (eight were dead) and 48 were R497K (23 were dead). The prognosis was significantly worse in *EGFR* wild type than in *EGFR* R497K polymorphism ($p = 0.0038$) (Fig. 5). In this cohort, pathological stages (stage I, $n = 11$, stage II, $n = 14$, stage III–IV, $n = 33$, $p = 0.0445$) but not gender (male, $n = 42$ vs. female, $n = 16$, $p = 0.9103$) was a prognostic factor. However, multi-variate analysis showed none of them was a prognostic factor.

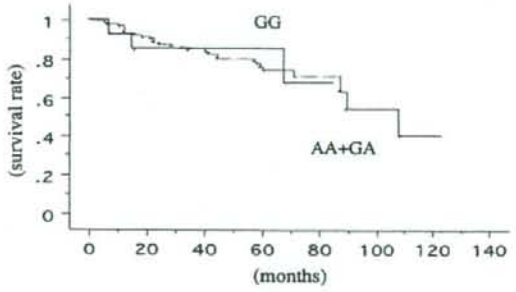

Fig. 3 The overall survival of node-negative lung cancer patients was studied in reference to the *EGFR* (R497K) status. There was no difference of survival between the patient with EGFR wild type (GG) ($n = 14$, 3 were dead) and the patient with R497K *EGFR* (GA or AA) ($n = 119$, 28 were dead) (Log-rank test, $p = 0.8882$)

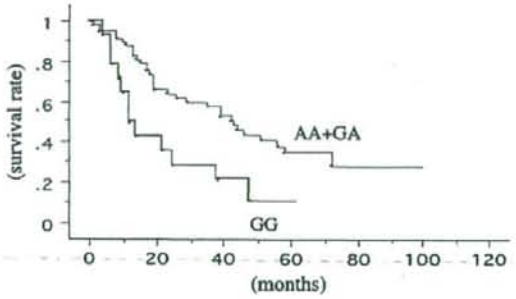

Fig. 4 The overall survival of node-positive lung cancer patients was studied in reference to the *EGFR* (R497K) status. The patients with EGFR wild type (GG) ($n = 14$, 12 were dead, median follow up = 21.7 months) had significantly worse prognosis than the patients with R497K *EGFR* (GA or AA) ($n = 59$, 33 were dead, median follow up = 42.7 months) (Log-rank test, $p = 0.0072$) (relative risk 2.4, 1.229–4.689)

Relationship between clinical courses of lung cancer patients treated with gefitinib and *EGFR*

The overall survival of gefitinib treated lung cancer patients from Nagoya City University, with follow-up through March 1, 2008, was studied in reference to the *EGFR* polymorphism status. Of 206 patients from Nagoya City University, 27 were treated with gefitinib therapy. Total 46 gefitinib treated patients were investigated the R497K polymorphism statuses. In this analysis, 38 patients had *EGFR* polymorphism (AG or GG). The prognosis after gefitinib therapy was not significantly different between *EGFR* wild type patients (GG, 5/8 were dead) and *EGFR* polymorphism patients (AG+GG; 28/38 were dead) ($p = 0.3100$) (Fig. 6).

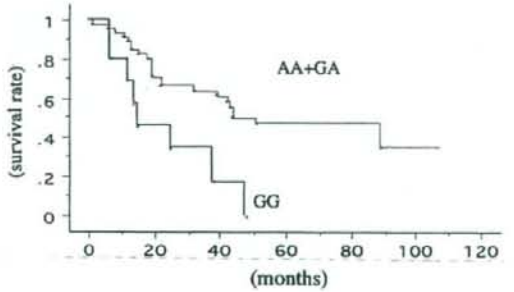

Fig. 5 The overall survival of adjuvant or neo-adjuvant chemo-untreated lung cancer patients was studied in reference to the *EGFR* (R497K) status. The patients with EGFR wild type (GG) ($n = 10$, 8 were dead, median follow up = 23.7 months) had significantly worse prognosis than the patients with R497K *EGFR* (GA or AA) ($n = 48$, 23 were dead, median follow up = 55.1 months) (Log-rank test, $p = 0.0038$)

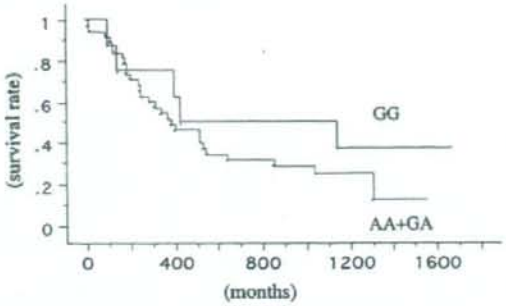

Fig. 6 The overall survival of 46 gefitinib untreated lung cancer patients was studied in reference to the *EGFR* (R497K) status. There was no difference of survival between the patients with EGFR wild type (GG) ($n = 8$, 5 were dead) and the patients with R497K *EGFR* (GA or AA) ($n = 38$, 28 were dead) (Log-rank test, $p = 0.3100$)

Discussion

In the present study, we showed that the R497 polymorphism of EGFR in node-positive lung cancer patients who received curative surgery might account for a longer overall survival. Moreover, this polymorphism was shown to correlate with a better prognosis after platinum-based adjuvant treatment. Although the underlying mechanisms remain unclear, an attenuated ligand interaction and consequential signal transduction might be the main reason for the suboptimal function of this receptor variant (Moriai et al. 1994).

The quantification of certain intratumoral molecules involved in the targeting or metabolism of specific chemotherapeutic agents may be valuable in predicting their efficacies or toxicities in cancer patients. For example, patients with a higher intratumoral level of excision repair cross complementation group 1 (ERCC1), an enzyme involved in nucleotide excision repair, may have a higher resistance to cisplatin-based adjuvant therapy in NSCLC (Olaussen et al. 2006). Moreover, NSCLC patients with a higher class III beta tubulin may have a higher resistance to taxane chemotherapy (Dumontet et al. 2005).

In this report, the R497K *EGFR* SNP(exon 13) is not associated with somatic *EGFR*-TK mutation. Approximately 563 *EGFR*-SNPs have been identified in human genome according to the National Cancer for Biotechnology information database. However, there are few studies examining associations between *EGFR* SNPs and human disease (Shintani et al. 1999; Kang et al. 2005; Fukushima et al. 2006; Zhang et al. 2006; Wang et al. 2007; Liu et al. 2008). In this study, we detected a polymorphism in exon 13 of the *EGFR*-extracellular domain, which changed amino acid Arg (R) to Lys (K), and the K allele seems to decrease the activity of EGFR (Moriai et al. 1994). Previous reports suggested that *EGFR* R497K polymorphism was weakly associated with gefitinib response (Liu et al. 2007). However, in our Japanese cohort, *EGFR* R497K was not associated with response to gefitinib. Although the survival curve of R497K showed higher than *EGFR* wild type (G/G) in our data, the larger number would help to determine the correlation between the R497K polymorphism and gefitinib sensitivity.

Previous report showed that patients with 497 Arg/Arg genotype tended to have a higher risk of local recurrence in chemo-treated rectal cancer patients (Zhang et al. 2005; Brandt et al. 2006). The patients with Arg/Arg genotype showed the highest risk of disease-specificity mortality and none of the patients with the Lys/Lys genotype died throughout the follow-up period of head and neck cancer treated with chemoradiation (Bandres et al. 2007). The mechanism through which the variant human EGFR R497K may account for lower local failures after chemotherapy is unknown (Zhang et al. 2005). A study with Chinese hamster ovary cells, the variant EGFR 497K cell line, showed an attenuated growth response to EGF and transforming growth factor-α, and a reduced induction of the proto-oncogenes fos, jun, and myc (Moriai et al. 1994). It was suggested that the amino acid substitution in the extracellular domain might modulate ligand binding and transmembrane signaling to the intracellular domain (Zhang et al. 2005). Thus, variant EGFR receptor may be less efficient in the recruitment of intracellular substrates and/or cause downstream activation of alternative signaling pathways with decreased proto-oncogene induction or growth stimulation, affecting chemosensitivity. Shintani et al. (1999) demonstrated that another *EGFR*-SNP at position 2073 was correlated with truncated *EGFR* transcription, which might interfere with EGFR three-dimensional structure and EGFR expression.

In summary, R497 polymorphism of EGFR in node-positive lung cancer patients had a better overall survival. R497K*EGFR* polymorphism might be associated with favorable prognosis of advanced lung cancers.

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Conflict of interest statement None declared.

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