Articles

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with EGFR TKIs.[14,15] We proposed that the absence of any survival advantage conferred by gefitinib monotherapy in previous studies[16–18] is due at least in part to a lack of patient selection, and that gefitinib would confer a survival advantage compared with platinum doublet chemotherapy in a first-line setting if eligible patients were selected on the basis of EGFR mutation status. To address this issue, we did a phase 3 trial that compared gefitinib with cisplatin plus docetaxel in patients with an EGFR mutation.

Methods

Patients

This study (WJTOG 3405) was a multicentre, randomised, open-label, phase 3, trial of first-line treatment with gefitinib versus cisplatin plus docetaxel for patients with advanced or recurrent non-small-cell lung cancer (NSCLC) harbouring an activating mutation of the EGFR gene. We recruited patients between March 31, 2006, and June 22, 2009, at 36 centres in Japan. All centres were members of the West Japan Oncology Group (WJOG), which is a Japanese non-profit organisation for oncological clinical trials (formerly the West Japan Thoracic Oncology Group, or WJTOG).

Initially, only patients with postoperative recurrence were eligible, because these surgical specimens were expected to ensure good sample quality. However, because of the initial slow accrual, the protocol was amended on July 10, 2006, to include patients with stage IIIB/IV disease. Patients were eligible if they had histologically or cytologically confirmed NSCLC, harbouring activating EGFR mutations (either exon 19 deletion or L858R in exon 21), were aged 75 years or younger, had WHO performance status 0–1, had measurable or non-measurable disease according to the Response Evaluation Criteria in Solid Tumours (RECIST), and had adequate organ function. Patients with postoperative recurrence, treated with adjuvant therapy other than cisplatin plus docetaxel, were included when the interval between the end of adjuvant chemotherapy and registration exceeded 6 months for platinum-doublet
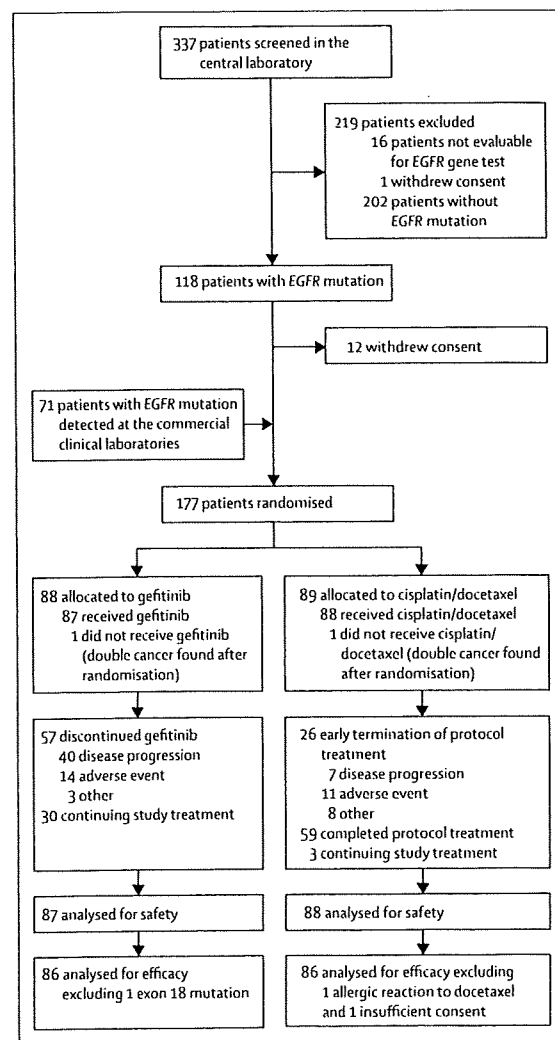

Figure 1: Trial profile

	Gefitinib (N=86)	Cisplatin plus docetaxel (N=86)
Sex		
Male	27	26
Female	59	60
Age (years; median; range)	64·0 (34–74)	64·0 (41–75)
Histological type		
Adenocarcinoma	83	84
Adenosquamous carcinoma	0	1
Squamous-cell carcinoma	1	0
Non-small-cell lung cancer; not otherwise specified	2	1
Smoking history		
Never	61	57
Former/current	25	29
Performance status		
0	56	52
1	30	34
Stage		
Postoperative recurrence	35	36
With postoperative adjuvant chemotherapy	19	23
Without postoperative adjuvant chemotherapy	16	13
IIIB	10	9
IV	41	41
EGFR mutation		
Exon 19 deletion	50	37
L858R	36	49

Table 1: Demographic and baseline characteristics of the modified intention-to-treat population

therapy and more than 1 month for oral tegafur plus uracil therapy. Patients were not eligible if they had received previous drug therapy that had targeted EGFR, had a history of interstitial lung disease, severe drug allergy, active infection or other serious disease condition, symptomatic brain metastases, poorly controlled pleural effusion, pericardial effusion or ascites necessitating drainage, active double cancer, or severe hypersensitivity to drugs containing polysolvate 80. Patients in pregnancy or lactation, or whose participation in the trial was judged to be inappropriate by the attending doctor, were not eligible. All patients provided written informed consent. Study approval was obtained from independent ethics committees at every institution. The study was undertaken in accordance with the Declaration of Helsinki.

Procedures

Patients were randomly assigned in a 1:1 ratio to receive gefitinib (250 mg/day, administered orally), or docetaxel (60 mg/m², administered intravenously over a 1 h period) followed by cisplatin (80 mg/m², administered intravenously over a 90-min period), with adequate hydration, in cycles of once every 21 days for three to six cycles.Treatment continued until progression of the disease, development of unacceptable toxic effects, a request by the patient to discontinue treatment, serious non-compliance with the protocol, or completion of three to six chemotherapy cycles. Further therapy after progression of the disease was at the physician's discretion. The primary endpoint was progression-free survival. Secondary endpoints included overall survival and response rate. Tertiary endpoints were disease control rate, safety, and mutation-type-specific survival.

Initially, patients were screened for *EGFR* mutation in a central laboratory at the Department of Molecular Diagnostics, Aichi Cancer Centre Hospital, Nagoya, Japan. The exon 19 deletion mutation was screened by fragment analysis and the L858R point mutation was screened by the Cycleave method, as described previously,[19] followed by confirmation by direct sequencing. On Feb 16, 2008, the protocol was amended to allow outsourcing of *EGFR* genetic testing from each institution to commercial clinical laboratories, either at SRL in Tokyo (direct sequencing), Mitsubishi Chemical Medience in Tokyo (peptide nucleic acid-locked nucleic acid PCR clamp[20]), or BML in Tokyo (PCR invader[21]), as this amendment would further facilitate patient accrual. The sensitivity of direct sequencing was anticipated to be less than that of other methods; however, false negativity was not a problem in this trial, since patients judged to lack EGFR mutations were not randomly allocated to a treatment.

Progression-free survival was assessed from the date of randomisation to the earliest sign of disease progression as determined by CT or MRI imaging using RECIST criteria, or death from any cause. Overall survival was assessed from the date of randomisation until death from any cause. Tumour response was assessed every 2 months
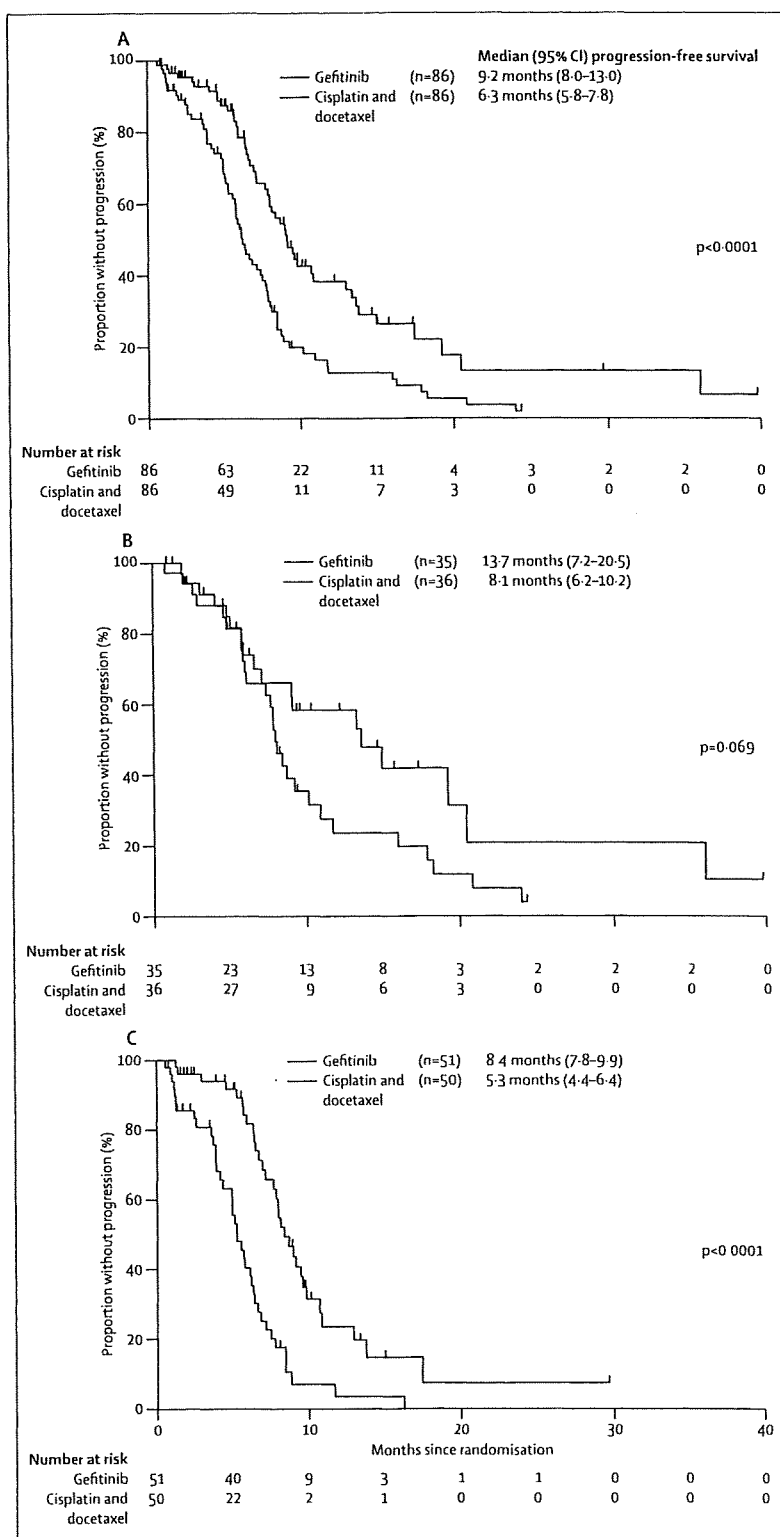

Figure 2: Progression-free survival in the overall population (A), in patients with postoperative recurrence (B), and in patients with stage IIIB/IV disease (C)
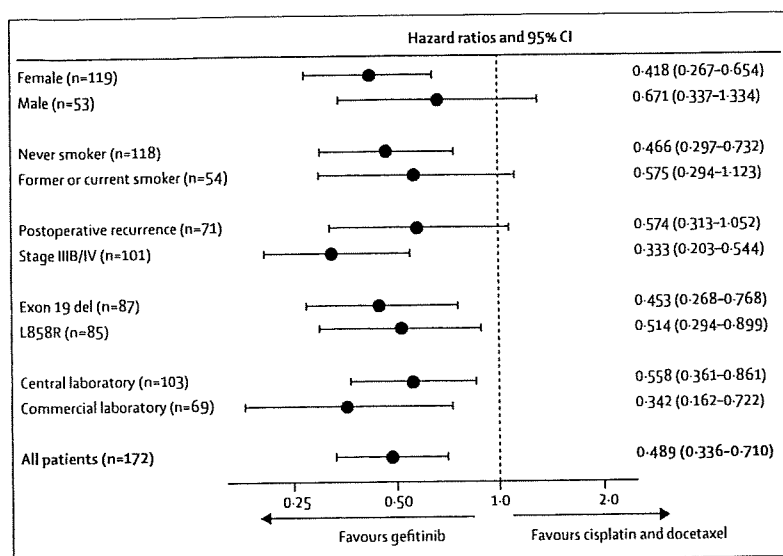
Figure 3: Hazard ratios for progression-free survival using subgroup analysis in the overall population
The shaded band represents the 95% CI of the hazard ratio for the overall population of patients.

	Univariate analysis		Multivariate analysis	
	HR (95% CI)	p	HR (95% CI)	p
Group (gefitinib/cisplatin plus docetaxel)	0·489 (0·336–0·710)	0·0002	0·258 (0·385–0·575)	<0·0001
Sex (male/female)	0·935 (0·625–1·398)	0·742	0·628 (0·361–1·092)	0·099
Age (<65 years /≥65 years)	1·091 (0·757–1·572)	0·641	1·183 (0·813–1·721)	0·380
Smoking history (never/former or current)	0·801 (0·541–1·186)	0·268	0·646 (0·378–1·105)	0·111
Stage (recurrence/IIIB–IV)	0·463 (0·220–0·976)	0·043	0·433 (0·290–0·649)	<0·0001
Mutation (exon 19 del/L858R)	1·001 (0·694–1·444)	0·996	1·135 (0·777–1·658)	0·514

Table 2: Univariate and multivariate analysis of progression-free survival

during the first year after randomisation, every 3 months between 12 and 18 months, and thereafter the interval of assessment was at the physician's discretion. Safety and tolerability were assessed according to National Cancer Institute Common Terminology Criteria (CTC) for Adverse Events, version 3.0. All events were confirmed via source-document verification at site visits to each participating institution by members of the WJOG data centre and the investigators.

Randomisation and masking

The investigator provided the necessary information to personnel at the WJOG data centre by fax. After an eligibility check, patients were allocated at the WJOG data centre to each treatment group using a desktop computer programmed for the minimisation method.[21] In this way, patient allocation was concealed from the investigator.

Because of the nature of treatment in each group, the study was open label. Stratification factors were: institution; postoperative adjuvant chemotherapy (presence vs absence); interval between surgery and recurrence (≥1 vs

<1 year) for patients with postoperative recurrent disease; and institution; stage (IIIB vs IV); and sex (male vs female) for patients with stage IIIB/IV disease.

Statistical analysis

In previous studies the progression-free survival of patients harbouring EGFR mutations and treated with gefitinib was reported as 12·6 months,[15] compared with 6·6 months for patients harbouring EGFR mutations treated with carboplatin plus paclitaxel.[11] Assuming a progression-free survival for gefitinib and platinum doublet chemotherapy of 12·5 and 7 months, respectively, would yield a hazard ratio (HR) of 0·56. Taking this HR into consideration, 146 patients would be required to achieve 90% power to show superiority with α=0·05 (two-sided). Therefore, sample size was initially set at 200 patients. While this trial was ongoing, the results of the Iressa Pan-Asia Study (IPASS) were presented at the annual meeting of the European Society for Medical Oncology (Stockholm, Sweden, Sept 12–16, 2008), and were later published.[14] Subgroup analysis of patients with EGFR mutations using about a third of the patients showed that the HR of gefitinib compared with carboplatin plus paclitaxel for progression-free survival was 0·48. Similarly, the HR of gefitinib compared with carboplatin plus paclitaxel for progression-free survival in patients with EGFR mutations was 0·36 in the study done by the North East Japan (NEJ) 002 Gefitinib Study Group, which was presented at the annual meeting of the American Society of Clinical Oncology (Orlando, FL, USA, May 29–June 2, 2009).[15] NEJ 002 was a phase 3 trial that analysed 198 patients with EGFR mutation randomised either to gefitinib or carboplatin plus paclitaxel. 177 patients had been randomised in our trial as of June 13, 2009, and 79 events had been noted during the regular monitoring done in March, 2009. The number of events needed to detect a conservative HR of 0·48 was calculated to be 78, based on normal approximation of the logarithm of the hazard ratio under α=0·05 (two-sided) and 90% power. Therefore, further accrual of patients was considered to be futile and potentially unethical. Although interim analysis was originally planned to analyse progression-free survival, this analysis was not done. Instead, the steering committee held on June 13, 2009, proposed the amendment of the sample size and the final analyses be done using available data. This proposal was approved by the independent data and safety monitoring committee on Aug 28, 2009. The data were locked on June 30, 2009. Patient follow-up for safety and survival will continue until 1·5 years after the last patient entry, as originally described in the study protocol.

Progression-free and overall survival were analysed for the modified intention-to-treat population as defined previously.[16] They were analysed using the Kaplan-Meier method, and were compared using the log-rank test. Hazard ratios in the overall population and in patient

subsets were calculated using the Cox proportional hazards model. The χ^2 test was used to compare proportions. Differences were considered significant at a two-sided p value of 0·05 or less. All statistical analyses were done with SAS version 9.1. This study is registered with UMIN (University Hospital Medical Information Network in Japan), number 000000539.

Role of the funding source

There was no sole study sponsor for this trial. The WJOG designed and did the trial independently of any pharmaceutical company. The report was written by the corresponding author, who had unrestricted access to the study data and is responsible for the accuracy and completeness of the reported analyses. The corresponding author had final responsibility for the decision to submit for publication.

Results

118 patients were positive for EGFR mutation at the central laboratory, 106 of whom were randomly allocated a treatment together with 71 patients with EGFR mutations who were tested at the commercial laboratories, giving a modified intention-to-treat population of 172 patients (figure 1). Baseline characteristics were well balanced between the two treatment groups (table 1), with the exception that the gefitinib group had an excess of exon 19 deletion mutations (50 of 86; 58·1%) compared with the cisplatin plus docetaxel group (37 of 86; 43·0%). Most of the patients had adenocarcinoma. 71 of 172 (41·3%) patients had postoperative recurrent disease, and 54 of 172 (31·4%) of the patients had a history of smoking. At the data collection cut-off time, the median follow-up was 81 days (range 74–1253 days), the median exposure to gefitinib was 165 days (range 22–1100 days), and the median number of cycles of cisplatin plus docetaxel chemotherapy was four, or 64 days (range one to six cycles, or 1–106 days).

Median progression-free survival was 9·2 months (95% CI 8·0–13·9) in the gefitinib group and 6·3 months (5·8–7·8) in the cisplatin plus docetaxel group (p<0·0001; figure 2A). Gefitinib treatment resulted in significantly longer progression-free survival than cisplatin plus docetaxel (HR 0·489; 95% CI 0·336–0·710; p<0·0001). Progression-free survival can be affected by the schedule of clinic visits and the interpretation of evidence of disease progression. We were able to confirm that the time schedule for clinic visits was almost the same in the two treatment groups (data not shown). In our trial, 71 patients had postoperative recurrent disease, and the remaining 101 patients had stage IIIB/IV disease. In both patient subsets, progression-free survival in the gefitinib group was longer than that in the cisplatin plus docetaxel group (figure 2B, 2C), although this was not a pre-specified analysis and was non-significant for those patients with postoperative recurrence. We noted that curves for each treatment group in the postoperative recurrence

subgroup (figure 2B) overlapped during the first 6 months, while the separation was clear during this time in the stage IIIB/IV group (figure 2C).

Patients treated with gefitinib had better progression-free survival than patients treated with cisplatin plus docetaxel in all subgroup analyses (figure 3). Additionally, gefitinib was better than cisplatin plus docetaxel, irrespective of where EGFR genetic testing was done. Exploratory analyses for progression-free survival showed that, in addition to the treatment group, patients with postoperative recurrent disease had a significantly better prognosis than those with stage IIIB/IV disease (table 2). We did a pre-planned comparison of exon 19 deletion with L858R in each treatment group. As shown in figure 4, mutation type was not prognostic. Therefore,
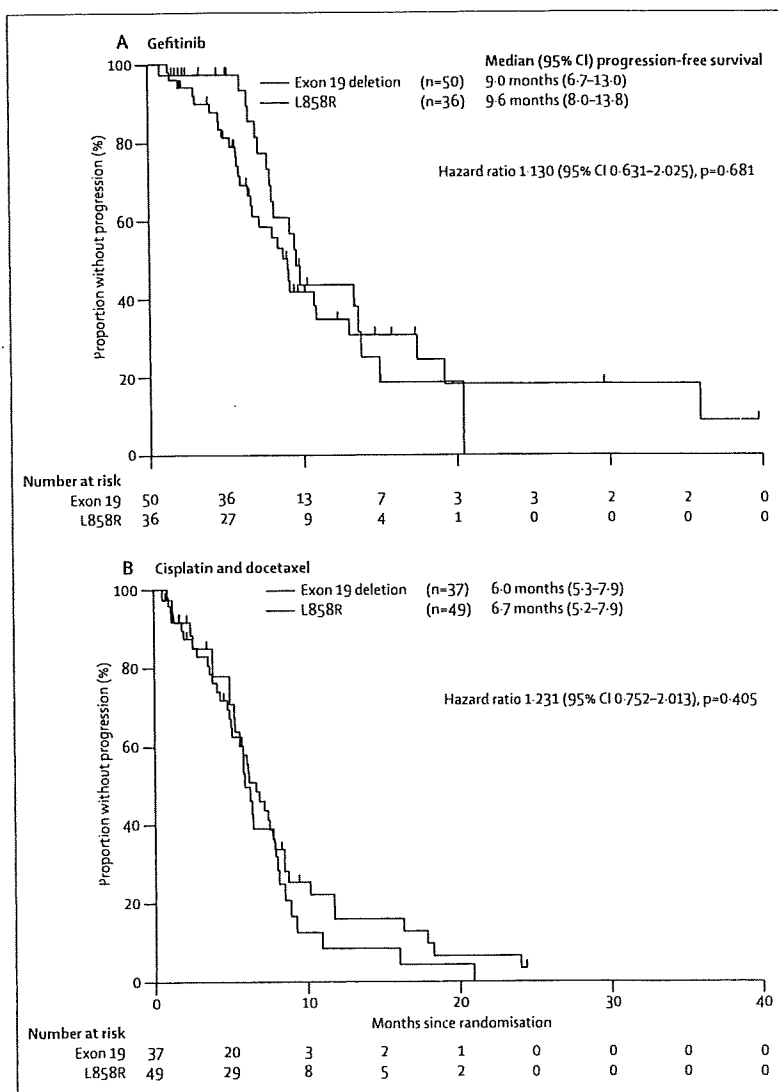

Figure 4: Progression-free survival in (A) the gefitinib group and (B) the cisplatin plus docetaxel group according to type of the EGFR mutation
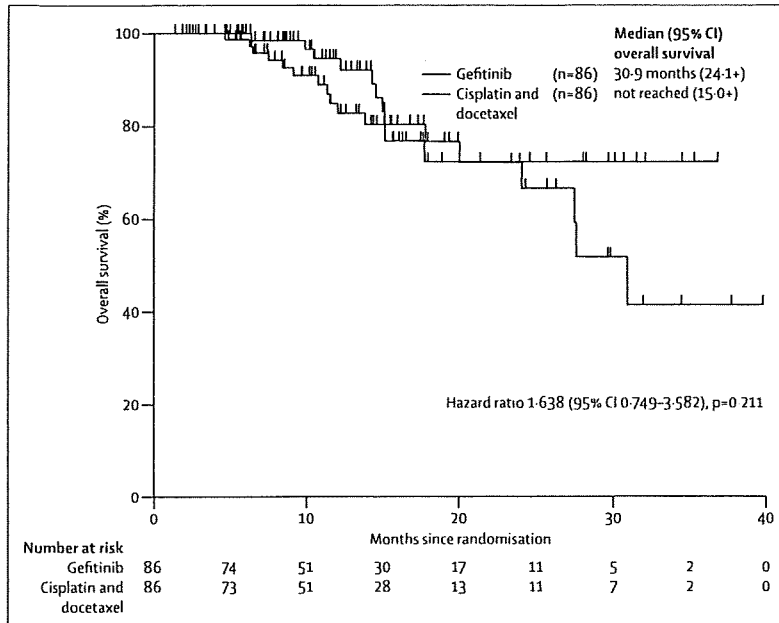
Figure 5: Overall survival in the overall population

See Online for webappendix

	Gefitinib (n=87)		Cisplatin plus docetaxel (n=88)	
	All	CTC grade ≥3	All	CTC grade ≥3
Non-haematological toxicity				
Rash*	74	2	7	0
AST*	61	14	17	1
ALT*	61	24	35	2
Dry skin*	47	0	3	0
Diarrhoea	47	1	35	0
Fatigue*	34	2	73	2
Paronychia*	28	1	1	0
Stomatitis	19	0	13	0
Nausea*	15	1	83	3
Constipation*	14	0	39	0
Alopecia*	8	0	67	0
Sensory disturbance*	7	1	23	0
Haematological toxicity				
Leucocytopenia*	13	0	82	43
Thrombocytopenia*	12	0	29	0
Neutropenia*	7	0	81	74
Anaemia*	33	0	79	15

ALT=alanine aminotransferase. AST=aspartate aminotransferase. CTC=National Cancer Institute Common Terminology Criteria. *p<0·001.

Table 3: Adverse events occurring in more than 10% of either of the treatment groups listed according to incidence in the gefitinib group

imbalance of mutation types was not likely to affect the interpretation of the overall results.

The objective response rate in the overall population with measurable disease (n=117) was 62·1% (36 of 58 patients) in the gefitinib group and 32·2% (19 of

59 patients) in the cisplatin plus docetaxel group (p<0·0001). The difference was significant (29·9%, 95% CI 12·6–47·1%; p<0·0001). The disease control rate was also higher in the gefitinib group (54/58, 93·1%) than in the cisplatin plus docetaxel group (46/59, 78·0%; difference in disease control rate 15·1%, 95% CI 2·7–27·6, p=0·020; webappendix). Because of frequent and detailed postoperative follow-up, which is standard practice in Japan, only 28 of 71 patients were found to have recurrent disease that met criteria for RECIST—ie, greater than 1 cm in the largest diameter. At the data cut-off, only 27 patients (15·7%) had died. Therefore, data for overall survival were immature, with follow-up still ongoing; 17 events (deaths) in the gefitinib group versus 10 events in the chemotherapy group—with an HR for gefitinib of 1·638 (95% CI, 0·75–3·58; figure 5). 51 patients in the chemotherapy group received an EGFR-TKI after they completed the study; 17 patients in the gefitinib group received post-protocol platinum doublet chemotherapy.

Adverse events occurring in more than 10% of either of the treatment groups are listed (table 3). The most common adverse event in the gefitinib group was skin rash followed by liver dysfunction, dry skin, and diarrhoea. However, adverse events with CTC grade 3 or more were infrequent, with the exception of liver dysfunction. By contrast, the most common adverse events in the cisplatin plus docetaxel group, which occurred in more than half of patients, were nausea, myelosuppression, fatigue, and alopecia.

Other potentially treatment-related toxicities included allergic reaction (one in gefitinib group, four in cisplatin plus docetaxel group) and oedema (one in gefitinib group, seven in the cisplatin plus docetaxel group). Two patients in the gefitinib group developed interstitial lung disease. There was one treatment-related death in the gefitinib group due to interstitial lung disease; there were no deaths in the cisplatin plus docetaxel group. There were no other serious adverse events.

Discussion

Our results show that first-line treatment with gefitinib conferred longer progression-free survival than treatment with cisplatin plus docetaxel in a molecularly defined (ie, EGFR mutation positive) group of patients with NSCLC.

In the IPASS study for patients with lung adenocarcinoma with no or former light smoking history, the progression-free survival of patients treated with gefitinib was significantly longer.[5] However, the curves crossed at the 6-month timepoint (initially chemotherapy was better, while gefitinib was better later). Molecular analysis for about a third of the patients suggested that the benefit of gefitinib was limited to patients with EGFR mutations with an HR of 0·48 (95% CI 0·36–0·64) and that gefitinib treatment was detrimental for patients without mutations (HR 2·85).[5] This result might seem similar to ours; however, the primary objective of the IPASS study was to assess gefitinib treatment in clinically selected patients,

Patient group		N	Median progression-free survival (months)			Median overall survival (months)	
			Gefitinib	Chemotherapy	HR (95% CI)	Gefitinib	Chemotherapy
Non-randomised pooled analysis							
I-CAMP[11]	Japanese, EGFR mutation	148	10·7	6·0	0·35 (0·23–0·52)	27·7	25·7
Subset analyses of the phase 3 trials for patients selected according to clinical backgrounds							
IPASS[15]	East Asian, light-non-smoker, adenocarcinoma	261	9·5	6·3	0·48 (0·36–0·64)	~20	~20
First SIGNAL[13]	Korean, non-smoker, adenocarcinoma	42	8·4	6·7	0·61 (0·31–1·22)	30·6	26·5
Phase 3 trials of patients selected according to EGFR mutation status							
NEJ 002[16]	Japanese, EGFR mutation	194	10·4	5·5	0·357(0·252–0·507)	28·0	23·6
WJTOG3405	Japanese, EGFR mutation	172	9·2	6·3	0·489 (0·336–0·710)	··	··

Table 4: Recent clinical trials assessing EGFR mutations as predictors of efficacy of gefitinib compared with chemotherapy

and not in molecularly selected patients, as was the case in our trial. In this context, a HR of 0·36 (95% CI 0·25–0·51)[26] for gefitinib compared with carboplatin plus paclitaxel in patients selected by EGFR mutation is highly relevant. Furthermore, our pooled analyses based on individual patient data from seven Japanese phase 2 studies that assessed prospectively the efficacy of gefitinib for patients with EGFR mutations (I-CAMP study)[11] and the pooled analysis of 1006 patients enrolled in a phase 3 trial of gefitinib[27] also showed similar progression-free survival of about 10 months for patients harbouring an EGFR mutation who were treated with gefitinb, while the median progression-free survival of patients treated with chemotherapy was 6·0 months (table 4).[11] These results strongly suggest that the presence of EGFR mutations, and not the clinical background of patients, determines clinical efficacy, and this knowledge should lead to molecularly based, personalised treatment of lung cancer.

Since the median duration of each treatment was quite different (165 days for gefitinib compared with 64 days for chemotherapy), one interpretation might be that a maintenance effect of gefitinib therapy contributed to the positive progression-free survival outcome, at least in part. Indeed, the progression-free survival curves of both groups in IPASS were initially similar, and then separate at about the time that chemotherapy stops. However, this was not the case in our trial, especially in patients with stage IIIB/IV disease. Furthermore, the SATURN[28] and the FAST-ACT[29] trials that tested maintenance erlotinib after chemotherapy showed that progression-free survival (both trials) and overall survival (SATURN) was prolonged. The benefit was much greater in patients with an EGFR mutation than in those without it in the SATURN trial.[28]

According to analyses of five US and European clinical trials that assessed first-line TKI treatment,[12] patients with the exon 19 deletion have a significantly longer progression-free and overall survival than patients with L858R (30·8 vs 14·8 months; p<0·0001). A similar trend was shown in a recent Spanish study.[11] In IPASS, the HR for progression-free survival for gefitinib versus chemotherapy was 0·38 (95% CI 0·25–0·56) in the subgroup of patients with exon 19 deletions, and 0·55 (95% CI 0·35–0·87) in the L858R mutation

subgroup, although a direct comparison between exon 19 deletion and L858R in the gefitinib group was not done.[10] However, recent Japanese trials, including I-CAMP[11] and this study, did not detect any difference. The reason for this discrepancy is not clear, although it might be attributable to ethnic differences or difference of EGFR-TKI used between study populations.

Two patients in the gefitinib group (2·3%) developed interstitial lung disease, one of whom died. This incidence was low compared with previous Japanese reports of 4·0% (59/1482)[31] and 3·5% (70/1976).[32] Selecting patients according to EGFR mutation status is expected to reduce the risk of interstitial lung disease, because risk factors for interstitial lung disease include smoking, male sex, and squamous histology, all of which are negative predictors of the presence of EGFR mutations.[31,32]

Our study indicates that EGFR genetic testing is feasible and should be done when possible. Although patients without EGFR mutations were not included in our study, potential harm of first-line gefitinib therapy compared with chemotherapy for patients without EGFR mutation shown in the IPASS[25] and the First-SIGNAL[11] study indicate the necessity of patient selection by EGFR mutation.

Clinical background might help identify patients who have a higher chance of carrying EGFR mutations. However, it should be noted that in a previous study,[9] eight of 37 (22%) patients with lung adenocarcinoma with a history of heavy smoking (>50 pack-years) harboured EGFR mutations.[9]

In conclusion, gefitinib significantly prolonged the progression-free survival of patients with NSCLC who carry EGFR mutations compared with cisplatin plus docetaxel. It is not yet known whether the prolonged progression-free survival conferred by gefitinib will translate into prolonged overall survival; we will continue to carefully follow-up our patients to determine its long-term effects. Considering the efficacy and toxicity of gefitinib, it is a reasonable option for the first-line treatment of patients with activating EGFR mutations.

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Phase I Dose-escalation and Pharmacokinetic Trial of Lapatinib (GW572016), a Selective Oral Dual Inhibitor of ErbB-1 and -2 Tyrosine Kinases, in Japanese Patients with Solid Tumors

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Objective: The Phase I dose-escalation study was conducted to evaluate the safety and pharmacokinetics of lapatinib (GW572016), a dual ErbB-1 and -2 inhibitor, in Japanese patients with solid tumors that generally express ErbB-1 and/or overexpress ErbB-2.
Methods: Patients received oral lapatinib once daily until disease progression or in an event of unacceptable toxicity.
Results: Twenty-four patients received lapatinib at dose levels of 900, 1200, 1600 and 1800 mg/day; six subjects enrolled to each dose level. The majority of drug-related adverse events was mild (Grade 1–2); the most common events were diarrhea (16 of 24; 67%), rash (13 of 24; 54%) and dry skin (8 of 24; 33%). No Grade 4 adverse event was observed. There were four Grade 3 drug-related adverse events in three patients (i.e. two events of diarrhea at 1600 and 1800 mg/day each and γ-glutamyl transpeptidase increase at 1800 mg/day). The maximum tolerated dose was 1800 mg/day. The pharmacokinetic profile of lapatinib in Japanese patients was comparable to that of western subjects.
Conclusions: Lapatinib was well tolerated at doses of 900–1600 mg/day in Japanese solid tumor patients. Overall, our findings were similar to those of overseas studies.

Key words: ErbB-1 — ErbB-2 — lapatinib — phase I — tyrosine kinase inhibitor

INTRODUCTION

Dysregulation of the human epidermal growth factor (ErbB) family of cell surface receptors has been noted in several solid tumors. Binding of extracellular ligand to ErbB receptors activates multiple intracellular signaling pathways that can promote tumor growth through processes, such as cell proliferation, differentiation and inhibition of apoptosis. ErbB-1 and ErbB-2 are implicated in the pathogenesis of several cancers (1), and their overexpression in epithelial tumors—including those of the lung, breast, head and neck, colon, stomach, ovary and prostate—often correlates with poor prognosis (2,3).

ErbB receptors present two rational targets for inhibition: blockade of the extracellular ligand-binding domain by monoclonal antibodies and inhibition of the intracellular tyrosine kinase domain by small molecules (4). Several anticancer agents target specific ErbB isoforms. For example, the small molecule tyrosine kinase inhibitors gefitinib (Iressa®) and erlotinib (Tarceva®) and the monoclonal antibody cetuximab (Erbitux®) all target ErbB-1 (5–7), and thus, they are indicated for the treatment of non-small cell lung cancer (NSCLC) and colorectal cancer (8,9). Furthermore, a monoclonal antibody directed against ErbB-2 (trastuzumab, Herceptin®) has been approved for patients with ErbB-2-overexpressing breast cancer (10). Sensitivity to some of these agents is strongly associated with the expression levels of ErbB-1 and -2 (2,3).

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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 down-stream effectors of cell proliferation and cell survival, respectively (14–17). Lapatinib inhibits tumor cell prolife-ration 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 $\sim$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 lapati-nib 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 evalu-ate the pharmacokinetics (PK) of repeated oral doses of lapa-tinib 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 cyto-logically confirmed solid tumors that are generally known to express EGFR and/or overexpress ErbB-2 (including colorec-tal 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 avail-able (19–40). Patients had to have normal function of major organs and adequate bone marrow, hepatic and renal func-tions 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 mito-mycin 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, intra-cavital administration, or any other relevant treatment; sys-tematic 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 guide-lines (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 break-fast, 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 incre-ments 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 per-formed 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 electro-cardiogram 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.

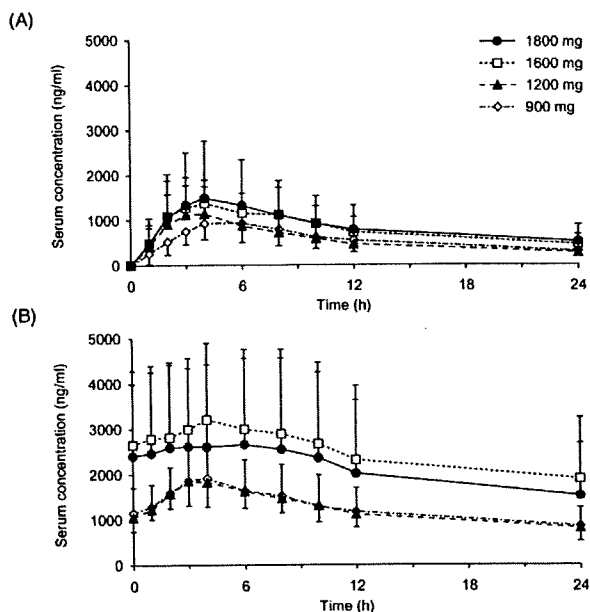[b] Day 1, AUC from 0 to infinity; Day 21, AUC from 0 to 24 h.

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

(A)

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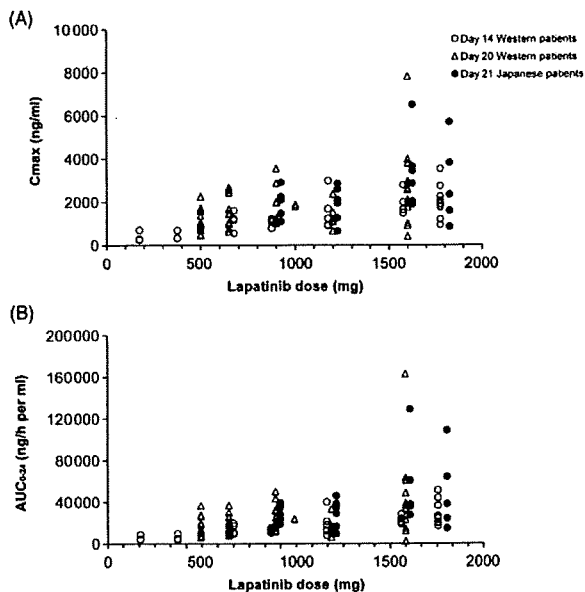
Figure 2. Relation between dose of lapatinib and exposure: comparison of (A) maximum serum concentration (C_{max}) and (B) area under the plasma drug concentration—time curve from 0 to 24 h (AUC_{0-24}) after dosing on Day 21 (our study, Japanese patients) and Days 14 and 20 (US studies, western patients).

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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Phase I Clinical and Pharmacokinetic Study of RAD001 (Everolimus) Administered Daily to Japanese Patients with Advanced Solid Tumors

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Objective: To determine the pharmacokinetics and safety of RAD001 (everolimus) in Japanese patients with advanced solid tumors.

Methods: An open-label, non-randomized, dose-escalation Phase I study of RAD001 administered continuously once daily in a 28-day cycle was performed. The study had a '3 + 3' design, with three patients recruited to each of three successive cohorts treated with RAD001 at 2.5, 5.0 or 10.0 mg/day.

Results: The pharmacokinetics of RAD001 in Japanese patients were similar to those previously determined in Caucasians. The drug safety profile was consistent with that of a mammalian target of rapamycin inhibitor. No dose-limiting toxicities were observed. One patient with esophageal cancer and one with gastric cancer treated with RAD001 at 10 mg/day showed marked tumor responses.

Conclusions: Treatment of Japanese cancer patients with RAD001 may be undertaken with the expectation that previously determined pharmacokinetic and safety profiles apply. The drug may hold promise for treatment of esophageal and gastric cancer.

Key words: Phase I study — pharmacokinetics — mTOR — RAD001 — everolimus

INTRODUCTION

Mammalian target of rapamycin (mTOR) is an intracellular protein kinase that mediates cellular responses to growth factors, nutrients and changes in energy status and thereby plays an important role in the regulation of cell growth, cell division and angiogenesis. It controls ribosome biosynthesis and the transcription of genes for many proteins that participate in the cell cycle, metabolism, nutrient transport or utilization, or the response to hypoxia. Various signaling defects upstream of mTOR, some of which are relatively common, have been identified in cancer cells and result in loss of cell growth control, unrestrained proliferation, tumor

angiogenesis, and other malignant characteristics. Defects in mTOR itself have not been identified in cancer, rendering this kinase both a well-situated and stable target for therapeutic intervention in cancers driven by defects in the mTOR signaling pathway (1–3).

RAD001 (everolimus) blocks the mTOR pathway by forming a complex with the immunophilin FK506-binding protein-12, which also binds mTOR with high affinity. This drug has exhibited antitumor activity with a variety of cancer cells both *in vitro* (4–9) and *in vivo* (10–12). In addition, the anticancer effects of RAD001 complement those of chemotherapy, radiation, hormonal agents and targeted therapeutics (13–15). RAD001 inhibits tumor growth

dependent on angiogenesis by inhibiting the production of angiogenic growth factors and thereby reducing the proliferation of neovascular endothelial cells (3). Phase I studies of RAD001 have shown sustained inhibition of mTOR activity in tumor tissue at oral doses of ≥ 20 mg weekly or 5–10 mg daily (16). Continuous daily dosing with RAD001 has been found to result in a more profound and sustained inhibition of mTOR than that achieved with an intermittent weekly schedule (17,18).

We have now performed a Phase I trial of RAD001 administered daily to Japanese patients with advanced solid tumors. The purpose of our study was to assess the pharmacokinetics, safety and tolerability of escalating oral doses of RAD001 in this patient population. An additional objective included evaluation of antitumor activity.

We herein report that RAD001 can be safely administered at daily doses up to 10 mg to Japanese patients with advanced solid malignancies. A dosage of 10 mg/day is recommended for further development.

PATIENTS AND METHODS

PATIENT POPULATION

Japanese individuals ≥ 20 years of age with a histologically confirmed diagnosis of an advanced tumor refractory to or unsuitable for existing standard therapy were included in the study if they had > 1 measurable lesion, a life expectancy of ≥ 3 months, and adequate or acceptable renal [serum creatinine concentration of $\leq 1.5 \times$ the upper limit of normal (ULN)], liver (serum bilirubin concentration of $\leq 1.25 \times$ ULN, serum transaminase activity of $\leq 3 \times$ ULN and serum albumin concentration of ≥ 3.5 g/dl) and bone marrow (absolute neutrophil count of $\geq 1500/\text{mm}^3$, platelet count of $\geq 1 \times 10^5/\text{mm}^3$ and hemoglobin concentration of ≥ 9 g/dl) function. Patients with tumors or metastases in the central nervous system, uncontrolled infection, gastrointestinal impairment disease, active bleeding diathesis, other concurrent or uncontrolled medical disease, or a history of coagulation disorders as well as those under treatment with strong inhibitors or inducers of isoenzyme CYP3A4 were excluded from the study. All subjects provided written informed consent to participation in the study, which was approved by the Institutional Review Board of each participating center and was performed in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines.

STUDY DESIGN

The study was an open-label, non-randomized, dose-escalation Phase I trial of RAD001 administered on a continuous once-daily schedule in a 28-day cycle to adult Japanese patients, with continuation of therapy after 28 days in the absence of progressive disease. The primary objective was to evaluate the tolerability/safety and dose-limiting toxicity (DLT) of RAD001, up to the dose level of 10 mg/day

which is being used in the global study. The study had a '3 + 3' design, with three patients recruited to each of three successive cohorts treated with RAD001 at 2.5, 5.0 or 10.0 mg/day. Patients were allowed to receive a higher RAD001 dose, at the investigator's discretion, if the higher dose had been confirmed as tolerable. Treatment was discontinued in the event of progressive disease, DLT, a dose delay of > 14 days (or > 42 days for hematologic DLTs), or withdrawal of consent. A DLT was defined as a hematologic (anemia, leukopenia, thrombocytopenia or neutropenia) or non-hematologic adverse event with a grade of ≥ 3 or a laboratory abnormality with a grade of ≥ 3 that occurred within the first 4 weeks of treatment and was suspected to be related to RAD001. Standard antiemetic prophylaxis and anti-hyperlipidemia therapy were allowed. Recruitment was permitted for Cohort 2 if DLTs were observed in 0/3 or $\leq 1/6$ patients in Cohort 1, and for Cohort 3 if DLTs were observed in 0/3 or $\leq 1/6$ patients in Cohort 2. DLTs in $\geq 2/6$ patients in Cohort 1 would result in study discontinuation; DLTs in $\geq 2/6$ patients in Cohort 2 or 3 would result in additional patient enrollment in Cohorts 1 and 2, respectively. The maximum-tolerated dose was defined as the dose at which two or more patients experienced a DLT in the first cycle.

ASSESSMENTS

Blood samples for pharmacokinetic analysis were collected on days 1 and 15 of cycle 1 at 0, 1, 2, 4, 6, 8 and 24 h after RAD001 administration. Blood samples for assessment of the trough concentration (C_{min}) of RAD001 were obtained immediately before administration of the next dose on days 2, 8, 11, 15 and 16 of cycle 1 and on day 1 of cycle 2 as well as at the end of the study. Pharmacokinetic parameters of RAD001 determined for each cohort included the maximum blood concentration (C_{max}), time of maximum concentration (t_{max}), area under the concentration-versus-time curve from time 0 to 24 h after drug administration (AUC$_\tau$, dosing interval) and apparent systemic clearance (CL/F). Drug safety and tolerability were assessed according to the NCI Common Terminology Criteria for Adverse Events (CTCAE) scale, version 3.0. Patients were monitored for adverse events throughout the study. Tumor volume was evaluated every 2 months and at the end of the study according to RECIST. Data were recorded for up to 28 days after discontinuation of treatment.

STATISTICS

The number of patients in each proposed cohort was based on the standard '3 + 3' design for dose-escalation studies. A total of 9–18 patients were planned to assess the safety and tolerability of RAD001, depending on observed toxicities. Descriptive statistics were used for evaluation of safety, efficacy and pharmacokinetic outcomes.

RESULTS

PATIENT CHARACTERISTICS

Between November 2005 and December 2006, nine patients with advanced, refractory solid tumors were enrolled in the study at the two participating centers (Kinki University School of Medicine and National Cancer Center Hospital East) (Table 1). The median age was 64 years (range, 49–74). All patients had received prior chemotherapy for their disease, and most of them had previously undergone cancer-related surgery. The median durations of RAD001 therapy were 57 days in the 2.5 mg/day cohort, 42 days in the 5 mg/day cohort and 98 days in the 10 mg/day cohort. Treatment was discontinued in all nine patients as a result of either progressive disease ($n = 4$), toxicities ($n = 2$), consent withdrawal ($n = 2$) or death ($n = 1$, hemorrhage). All patients were evaluable for drug safety and pharmacokinetics.

SAFETY

DLTs were not observed for any patient in the first cycle of treatment (28 days). Overall, the most common adverse events of all grades were thrombocytopenia (56% of patients), leukopenia (33%), anorexia (44%) and rash (44%) (Table 2). One patient with colon cancer and both lung and liver metastases was treated at the RAD001 dose of 5 mg/day experienced grade 2 pneumonitis after 142 days of therapy. The patient developed cough, and computed tomographic scan of the chest revealed new ground-glass opacities. The patient was hospitalized with PaO_2 of 72.7 mmHg.

Table 1. Patient characteristics

Characteristic	No. of patients
Sex	
Male	4
Female	5
Performance status (ECOG)	
0	5
1	4
Previous therapy	
Surgery	8
Chemotherapy	9
Radiotherapy	3
Tumor type	
Colorectal cancer	3
Lung cancer	3
Esophageal cancer	1
Gastric cancer	1
Thyroid cancer	1

The median (range) age was 64 (49–74) years. ECOG, Eastern Cooperative Oncology Group.

Table 2. Number of patients with adverse events in all courses thought to be attributable to RAD001

Adverse event	RAD001 dose (mg/day)						Total
	2.5 ($n = 3$)		5 ($n = 3$)		10 ($n = 3$)		
	G1/2	G3/4	G1/2	G3/4	G1/2	G3/4	
Thrombocytopenia	0	0	2	0	3	0	5
Leukopenia	1	0	2	0	0	0	3
Neutropenia	1	0	0	0	0	0	1
Anemia	0	0	0	0	1	0	1
Anorexia	1	0	2	0	1	0	4
Rash	0	0	1	0	3	0	4
Stomatitis	1	0	0	0	0	1	2
Nausea	1	0	0	0	1	0	2
Mucosal inflammation	0	0	0	0	2	0	2
Diarrhea	0	0	2	0	0	0	2
Fatigue	0	0	1	0	0	1	2
Weight decreased	1	0	0	0	1	0	2
Elevated ALT or AST	2	0	0	0	0	0	2
Hyperglycemia	0	0	0	0	0	1	1
Hemorrhage	0	0	0	0	0	1	1
Pneumonitis	0	0	1	0	0	0	1
Hypertension	1	0	0	0	0	0	1
Glucose tolerance impaired	0	0	1	0	0	0	1

Includes all adverse events occurring in two or more patients or were ≥Grade 2. G, grade; ALT, alanine aminotransferase; AST, aspartate aminotransferase.

Steroid treatment and discontinuation of RAD001 resulted in marked improvement of the patient within days. All toxicities of Grade 3 or 4 occurred at the dose of 10 mg/day, but none occurred in the first cycle and therefore did not qualify as DLTs. One patient with advanced esophageal carcinoma at a dose of 10 mg/day developed Grade 3 fatigue and stomatitis on day 58 and RAD001 was interrupted. The study drug was restarted on day 66 at a reduced dose of 5 mg/day. On day 71, the patient visited the hospital because of hemorrhage from the right supraclavicular tumor which was a metastatic focus. Although the patient was treated as an inpatient, the Grade 4 hemorrhage could not be controlled and the patient died on day 78. Since RAD001 markedly diminished the size of the patient's metastatic focus, the cause of death was hemorrhage from either the right supraclavicular metastatic focus or the enriched vessels. The study drug did not seem to be the direct cause of hemorrhage. The other two patients in the 10 mg/day cohort took RAD001 for >3 months. One patient with colorectal cancer was treated with 10 mg/day and experienced Grade 3 hyperglycemia on day 98. The patient was determined to have progressive disease on the same day. Another patient in the 10 mg/day cohort

did not have any Grade 3 or 4 toxicities and discontinued RAD001 due to disease progression on day 154.

PHARMACOKINETICS

Pharmacokinetic parameters of RAD001 are summarized in Table 3. The C_{max} of RAD001 was apparent 2 h after administration of a single dose of the oral drug (Table 3 and Fig. 1A). The C_{min} of RAD001 indicated that a steady state was attained after ~8 days of repeated once-daily oral dosing (Fig. 1B). Determination of the AUC_τ on days 1 and 15 revealed that the exposure to RAD001 achieved after multiple dosing was about twice that achieved after a single

dose (Table 3). On day 1, C_{max} and AUC_τ increased almost dose-proportionally. At steady state (day 15), C_{max} and AUC_τ increased with increment of dose but dose-proportionality was not clear due to large inter-individual variability in the 5 mg/day cohort.

TUMOR RESPONSE

Among seven patients evaluable for tumor response, obvious tumor shrinkage was observed in two patients treated at the dose level of 10 mg/day. A 60-year-old male with advanced esophageal carcinoma who had been treated with seven prior chemotherapy regimens started treatment with RAD001 at

Table 3. Pharmacokinetic parameters of RAD001

	RAD001 dose (mg/day)		
	2.5 ($n = 3$)	5 ($n = 3$)	10 ($n = 3$)
Day 1			
t_{max} (h)			
Median	1.98	1.00	2.00
Range	0.98–2.00	1.00–1.95	1.92–2.00
C_{max} (ng/ml)	15.1 ± 2.48	31.5 ± 3.40	49.4 ± 14.8
AUC_τ (ng h/ml)	85.2 ± 18.7	211 ± 50.0	401 ± 51.6
Day 15			
t_{max} (h)			
Median	1.92	1.98	2.02
Range	1.00–1.98	1.93–1.98	2.00–2.20
C_{max} (ng/ml)	16.8 ± 1.33	57.6 ± 17.6	65.9 ± 1.40
AUC_τ (ng h/ml)	134 ± 24.1	543 ± 189	711 ± 113
CL/F (l/h)	19.1 ± 3.26	9.94 ± 3.21	14.3 ± 2.23

Data are means ± SD unless indicated otherwise. t_{max}, time of maximum concentration; C_{max}, maximum blood concentration; AUC_τ, area under the concentration-versus-time curve from time 0 to 24 h after drug administration; CL/F, apparent systemic clearance.
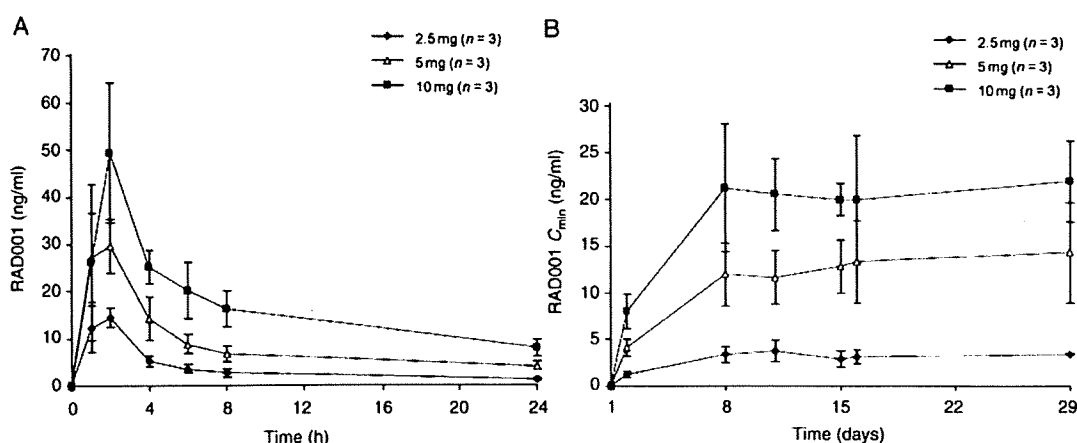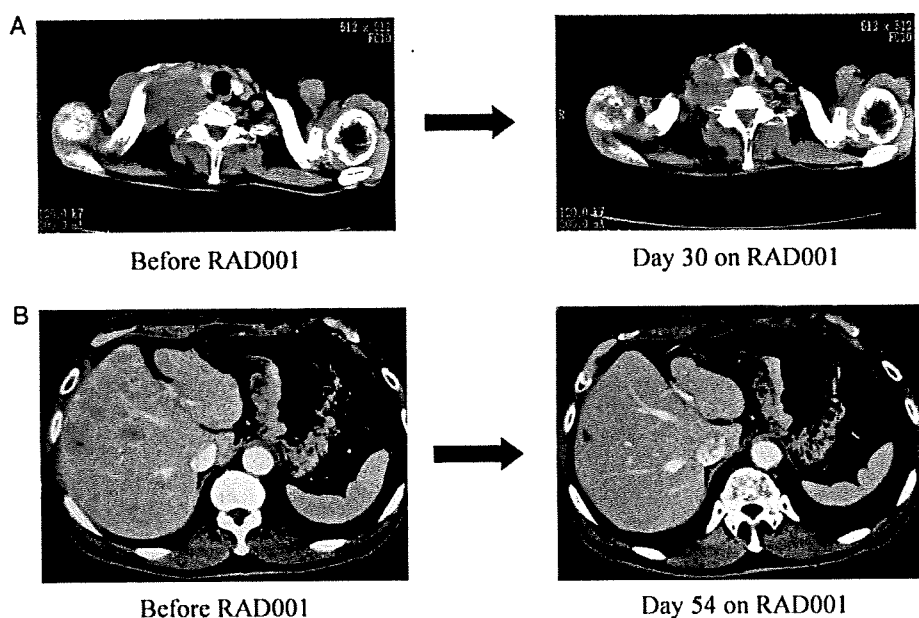

Figure 1. Pharmacokinetics of RAD001. (A) Blood concentration of RAD001 after administration of a single oral dose (2.5, 5 or 10 mg) on day 1 of cycle 1. Data are means ± SD. (B) Blood trough concentration (C_{min}) of RAD001 during continuous oral dosing for 29 days (cycle 1). Data are means ± SD.

Figure 2. Computed tomography images of tumor response to RAD001 treatment. (A) Shrinkage of metastases in supraclavicular lymph nodes in a patient with esophageal cancer. (B) Shrinkage of liver metastases in a patient with gastric cancer.

10 mg/day. After one cycle of RAD001 treatment, computed tomography revealed that lymph nodes with metastases in the right supraclavicular region had shrunk markedly (Fig. 2A). A 64-year-old male with gastric adenocarcinoma and liver metastases who had undergone four prior chemotherapy regimens showed a partial response to RAD001 that persisted for >4 months at the dose of 10 mg/day (Fig. 2B).

DISCUSSION

Evidence implicating the phosphatidylinositol 3-kinase—Akt—mTOR signaling pathway in the pathogenesis of a variety of malignancies has prompted the development of therapeutic strategies to modulate this pathway. RAD001 is an oral inhibitor of the mTOR pathway, and we have now performed a dose-escalation Phase I study of this drug in Japanese patients with advanced solid tumors in order to evaluate its safety and pharmacokinetics. Therapy with RAD001 at oral doses of up to 10 mg once daily was relatively well tolerated in the study subjects. Indeed, the safety and tolerability of RAD001 in the Japanese patients were similar to those observed in previous studies with larger populations of Caucasian patients, for whom the most common drug-related toxicities included rash, stomatitis and fatigue. Previous studies have reported that patients receiving RAD001 manifested hyperglycemia and hyperlipidemia, probably as a result of inhibition of mTOR-regulated glucose and lipid metabolism (16,18,19). Grade 3 hyperglycemia was observed in one patient treated with 10 mg/day, whereas hyperlipidemia was not observed in our study. One patient in our study developed pneumonitis of Grade 2, with this

condition having previously been identified as a potential class-related toxicity for mTOR inhibitors that should be monitored in clinical trials with these agents (16—20). However, the condition of pneumonitis in our study was reversible after discontinuation of RAD001 treatment. The pharmacokinetic profile of RAD001 in Japanese patients was also similar to that in Caucasian patients. RAD001 was absorbed rapidly, with the C_{max} being achieved as early as 1—2 h after oral administration. A recent Phase I study of RAD001 performed in Europe and the USA showed that the mean ($\pm$ SD) C_{max} in patients with advanced cancer was 32 $\pm$ 9 and 61 $\pm$ 17 ng/ml at daily doses of 5 and 10 mg, respectively, with a mean AUC_τ of 238 $\pm$ 77 and 514 $\pm$ 231 ng h/ml, respectively (16). These results for Caucasian patients are similar to those obtained here with Japanese patients, especially for the dose level of 10 mg/day (C_{max} of 65.9 $\pm$ 1.40 ng/ml and AUC_τ of 711 $\pm$ 113 ng h/ml. Given the limited number of patients in both studies, these results suggest that there are no substantial differences in the pharmacokinetics of RAD001 between the two populations. RAD001 has already undergone extensive clinical testing in the setting of renal and cardiac transplantation (21,22). Our present data are also supported by observations with 673 renal transplant patients who received RAD001 (23). This large cohort included 80% Caucasian patients and 2.5% patients of Asian origin with no significant differences in clearance of RAD001 being apparent between the Asian and Caucasian patients. The data from this study, combined with those from previous studies, suggest that the pharmacokinetic and safety data for RAD001 obtained in larger clinical trials with Caucasian patients are likely applicable to the Japanese population.