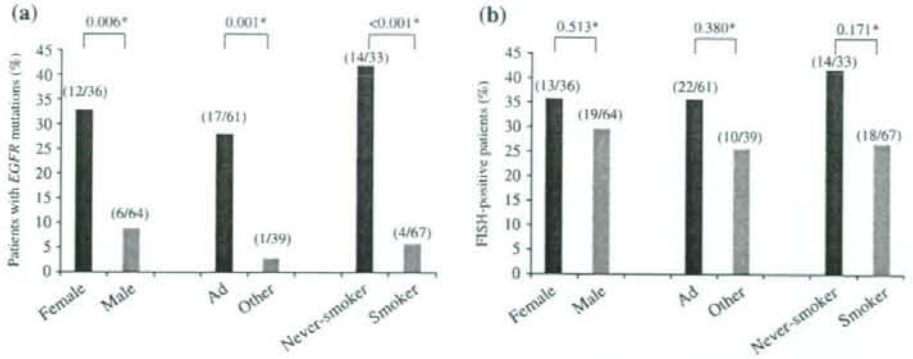

Fig. 1. Sex, tumor histology, and smoking status of patients with advanced non-small cell lung cancer and with either (a) epidermal growth factor receptor (*EGFR*) mutations or (b) a high *EGFR* copy number. Ad, adenocarcinoma. *P-values were determined by Fisher's exact test.

Table 4. Relationship between epidermal growth factor receptor (*EGFR*) mutation and either fluorescence *in situ* hybridization (FISH) status of *EGFR* amplification

Mutation status	FISH status		Gene amplification	
	Positive	Negative	Positive	Negative
Positive ($n = 18$)	8	10	4	14
Negative ($n = 82$)	24	58	2	80
P-value*		0.266		0.009

*Determined by Fisher's exact test.

positivity was not associated with sex, tumor histology, or smoking status (Fig. 1b). Although no relationship was apparent between *EGFR* mutation and FISH positivity (gene amplification or high polysomy), *EGFR* mutation and *EGFR* amplification were significantly associated (Table 4). The clinicopathological and genetic features of patients with *EGFR* mutations are shown in Table 5.

Overall survival. For the total patient population, the median overall survival was 12.3 months, with a 1-year survival rate of 51.7%. Univariate analysis revealed that overall survival was significantly longer in women, never-smokers, patients with a favorable PS, and those with *EGFR* mutations (Table 6; Fig. 2a). In contrast, no difference in overall survival was apparent between FISH-positive and FISH-negative patients (Table 6; Fig. 2b). We also carried out multivariate analysis to identify factors that contribute to overall survival, with covariates including clinicopathological and genetic factors (sex, smoking history, tumor histology, PS, *EGFR* mutation status, FISH status). Female sex and favorable PS were found to be independent prognostic factors (Table 6).

Responsiveness to epidermal growth factor receptor tyrosine kinase inhibitor treatment. Of the 53 patients treated with EGFR TKI, 40 individuals were assessable for objective response. Whereas the rate of response to EGFR TKI treatment for patients with *EGFR* mutations was significantly higher than that for those without such mutations (71.4 vs 11.5%, $P < 0.001$), there was no significant association between FISH status and responsiveness

Table 5. Clinicopathological and genetic features of patients with epidermal growth factor receptor (*EGFR*) mutations

No.	Age (years)	Sex	Smoking status	Histology	Response to EGFR TKI	Type of *EGFR* mutation		*EGFR* copy number
						Sequencing	ARMS	
1	72	F	Never	Ad	PR		L858R	Low trisomy
2	58	F	Never	Ad	PR	L858R	L858R	Gene amplification
3	81	F	Never	Ad	SD	L858R	L858R	High polysomy
4	72	F	Never	Ad	NE		L858R	Gene amplification
5	48	M	Smoker	Ad	SD		L858R	Low trisomy
6	67	F	Never	Ad	SD		L858R	Low trisomy
7	59	F	Never	Ad	PR		L858R	High polysomy
8	78	M	Smoker	Ad			L858R	High trisomy
9	71	F	Never	Ad	PR		L858R	Low polysomy
10	82	F	Never	Ad	PR	L858R	L858R	Low trisomy
11	67	F	Never	Ad		L858R	L858R	High polysomy
12	87	F	Never	Sq	PR	L858R	L858R	Low polysomy
13	78	M	Never	Ad			L858R	Gene amplification
14	56	F	Never	Ad	PR		(E746_A750)del	Low polysomy
15	63	M	Never	Ad	PD	(E746_A750)del	(E746_A750)del	Gene amplification
16	63	M	Smoker	Ad	PR		(E746_A750)del	Low polysomy
17	61	M	Smoker	Ad	PR	(E746_S752)del insV		Low trisomy
18	73	F	Never	Ad	PR	(E746_T751)del insS		High polysomy

Ad, adenocarcinoma; ARMS, amplification-refractory mutation system; NE, not evaluated; PD, progressive disease; PR, partial response; SD, stable disease; Sq, squamous cell carcinoma; TKI, tyrosine kinase inhibitor.

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Table 6. Univariate and multivariate analyses of prognostic factors for overall survival

Factor	Univariate analysis			Multivariate analysis		
	HR	95% CI	*P*-value	HR	95% CI	*P*-value
Sex (female/male)	0.54	0.32–0.91	**0.021**	0.55	0.32–0.93	**0.025**
Smoking history (never-smoker/smoker)	0.50	0.30–0.85	**0.011**			
Histology (adenocarcinoma/other)	0.64	0.39–1.05	0.077	0.68	0.40–1.14	0.141
ECOG PS (0/≥1)	0.44	0.24–0.79	**0.006**	0.48	0.29–0.86	**0.019**
EGFR mutation status (positive/negative)	0.52	0.28–0.97	**0.039**			
FISH status (positive/negative)	1.36	0.82–2.23	0.231	1.49	0.88–2.50	0.130

CI, confidence interval; ECOG, Eastern Cooperative Oncology Group; EGFR, epidermal growth factor receptor; FISH, fluorescence *in situ* hybridization; HR, hazard ratio; PS, performance status. Multivariate analysis was carried out using the stepwise method (include, <0.05; exclude, >0.2). Significant *P*-values are shown in bold.

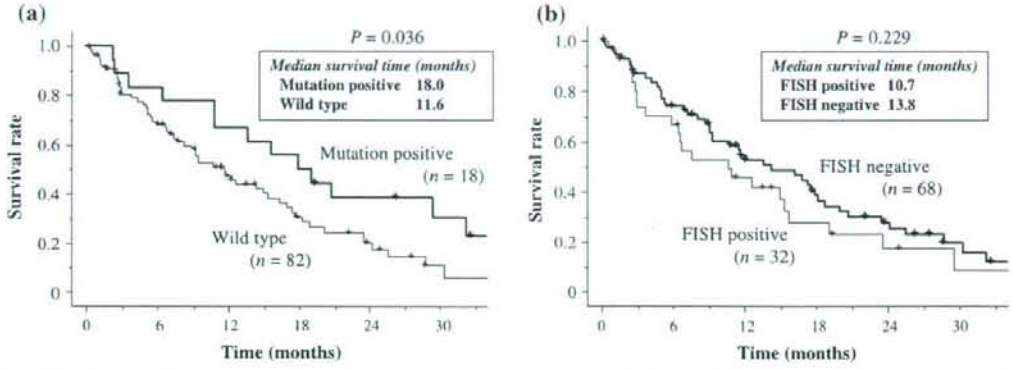

Fig. 2. Kaplan–Meier plots of overall survival in patients with advanced non-small cell lung cancer and either (a) with or without epidermal growth factor receptor (*EGFR*) mutations or (b) with or without a high *EGFR* copy number. FISH, fluorescence *in situ* hybridization.

to EGFR TKI (44.4 vs 29.0% for FISH-positive vs FISH-negative patients, respectively, $P = 0.437$).

Discussion

We have analyzed both *EGFR* mutation and *EGFR* copy number in paired tumor specimens as well as the relationship between these two types of *EGFR* alterations in advanced NSCLC. We used two methods to detect *EGFR* mutations, direct sequencing and Scorpion-ARMS, which identified eight and 16 mutations, respectively. Direct sequencing failed to detect 10 of the 16 mutations identified by Scorpion-ARMS. Of the 10 patients with *EGFR* mutations detected by Scorpion-ARMS alone, seven were assessable for an objective response to EGFR TKI, with five exhibiting a partial response and two having stable disease. Consistent with previous observations,[28–30] our data thus indicate that Scorpion-ARMS is more sensitive than direct sequencing for detection of the two major types of *EGFR* mutation that reflect responsiveness to EGFR TKI. It should be noted, however, that most polymerase chain reaction-based systems for mutation analysis, including Scorpion-ARMS, are able to detect only known *EGFR* mutations targeted by the designed primers. Indeed, two minor variants of deletion mutation in exon 19 were not identified by Scorpion-ARMS in the present study. Given the exclusion of recurrence after surgical resection in our study, most tumor specimens analyzed were obtained either by transbronchial lung biopsy or by percutaneous needle lung biopsy. The amount of tumor tissue obtained by these procedures is limited, but our results suggest that it is sufficient both for histopathological analysis and for the detection of *EGFR* mutations by Scorpion-ARMS in patients with advanced NSCLC.

Scorpion-ARMS identified three E746_A750 deletion mutations in exon 19 and 13 L858R point mutations in exon 21 in the present study. The frequency of the E746_A750 mutation detected by Scorpion-ARMS thus appeared low compared with that of the L858R mutation. Previous studies have shown that the incidence of the E746_A750 deletion is approximately the same as that of the L858R mutation.[10,12] The sensitivity of Scorpion-ARMS for detection of the E746_A750 deletion is equivalent to that for detection of the L858R point mutation. The low frequency of the E746_A750 deletion mutation in the present study is thus likely due to the small number of samples.

Previous studies have revealed a higher prevalence of *EGFR* mutations in East Asians than in Caucasians.[4,10–12,20,22,24,26,27,32–36] The prevalence of *EGFR* mutations in our Japanese cohort was low (18%) compared with values determined previously for East Asian populations. Given that most previous studies examined only individuals treated with EGFR TKI, patient selection based on clinical predictors might have led to an increase in the proportion of subjects with adenocarcinoma histology, a factor known to be associated with *EGFR* mutations. In contrast, our study was carried out with consecutive cases irrespective of EGFR TKI treatment. The relatively low proportion of patients with adenocarcinoma histology (61%) in our cohort is therefore consistent with the low prevalence of *EGFR* mutations. However, the FISH positivity of 32% in our study is similar to that in previous studies that adopted the same criteria, with values ranging from 31 to 48%.[22–24,26,27] Consistent with previous

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results,[1,7–9,12] *EGFR* mutations were significantly more frequent among women, never-smokers, and patients with adenocarcinoma in the present study. In contrast, neither *EGFR* amplification (analysis not shown) nor FISH positivity was associated with any such clinicopathological factor in our study, although the relationship between *EGFR* amplification and never-smoking status approached statistical significance ($P = 0.090$).

The relationship between *EGFR* mutation and FISH positivity (gene amplification or high polysomy) in NSCLC patients has remained unclear.[22–24,26,27] In the present study, we have demonstrated a significant relationship between *EGFR* mutation and *EGFR* amplification, but not between *EGFR* mutation and FISH positivity, in tumor specimens from patients with advanced NSCLC. *EGFR* mutant alleles were previously found to be amplified selectively, resulting in a high *EGFR* copy number, as detected by quantitative real-time polymerase chain reaction analysis.[12] *EGFR* amplification has also been shown to be acquired during invasive growth of lung adenocarcinoma with *EGFR* mutations.[37] Furthermore, recent studies have found that an increase in *EGFR* copy number is a relatively late event in NSCLC pathogenesis[38] and that *EGFR* mutation precedes *EGFR* amplification but not necessarily high polysomy.[37,39] These observations thus support the existence of a close association between *EGFR* mutation and *EGFR* amplification. We previously showed that *EGFR* mutation was significantly associated with *EGFR* amplification in human NSCLC cell lines and that endogenous EGFR expressed in such cell lines that manifested both of these *EGFR* alterations were activated constitutively as a result of ligand-independent dimerization.[25] However, the biological consequences of high polysomy for *EGFR* have not been elucidated. We did not find any cut-off value of high polysomy that was associated with *EGFR* mutation. We therefore propose that *EGFR* amplification, but not high polysomy, plays a key role in the pathogenesis of NSCLC and correlates with *EGFR* mutation.

We sought to determine whether *EGFR* mutation or *EGFR* copy number might affect overall survival of NSCLC patients. Previous studies of EGFR TKI have suggested that *EGFR* mutation is a favorable prognostic indicator for patients with NSCLC.[35,36] We also found that the survival time of patients with *EGFR* mutations was longer than that of those without them (18.0 vs 11.6 months, $P = 0.036$) in the univariate analysis. However, interpretation of this result requires that the effect of EGFR TKI on survival be taken into account, given that 83% (15/18) of patients with *EGFR* mutations were treated with EGFR TKI compared with only 46% (38/82) of those without such mutations. Indeed, analysis of survival after initiation of EGFR TKI treatment as a second-line or subsequent therapy revealed a survival time of 15.6 months for mutation-positive patients vs 6.0 months for mutation-negative patients in our study. It was therefore not possible to determine the prognostic significance of *EGFR* mutation for NSCLC patients. To clarify whether *EGFR* mutation is a predictor of sensitivity to EGFR TKI or a prognostic indicator for NSCLC patients, we are currently carrying out a phase III randomized study comparing platinum-based chemotherapy with gefitinib in chemotherapy-naive NSCLC patients with *EGFR* mutations. Patients with FISH-positive tumors tended to have a shorter survival time than did those with FISH-negative tumors (10.7 vs 13.8 months), although this difference was not statistically significant. This result is consistent with previous observations indicative of an association between high *EGFR* copy number and poor prognosis for certain malignancies, including NSCLC.[1,40]

In conclusion, we have analyzed both *EGFR* mutation and *EGFR* copy number in paired tumor specimens from patients with advanced NSCLC. We found that Scorpion-ARMS is more sensitive than direct sequencing for detection of *EGFR* mutations in small tumor specimens. Furthermore, we showed that *EGFR* mutation was significantly associated with *EGFR* amplification but not with FISH positivity. These observations warrant confirmation in further studies as well as exploration of the biological mechanisms of the relationship between *EGFR* mutation and *EGFR* amplification. The effects of *EGFR* mutation and *EGFR* copy number on clinical outcome in individuals with advanced NSCLC also warrant investigation in a prospective study.

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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 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.

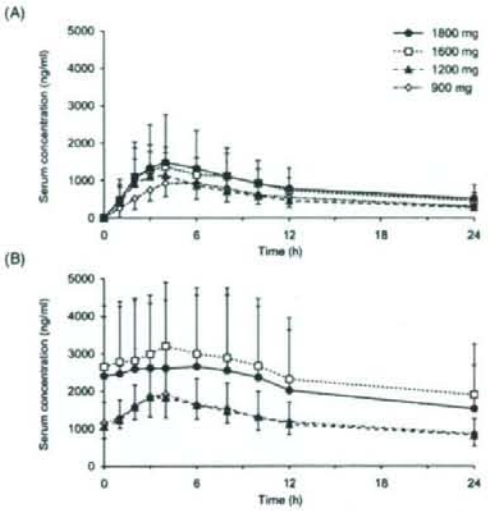

Figure 1. Serum concentrations of lapatinib at each dose level as detected on (A) Day 1 and (B) Day 21.

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

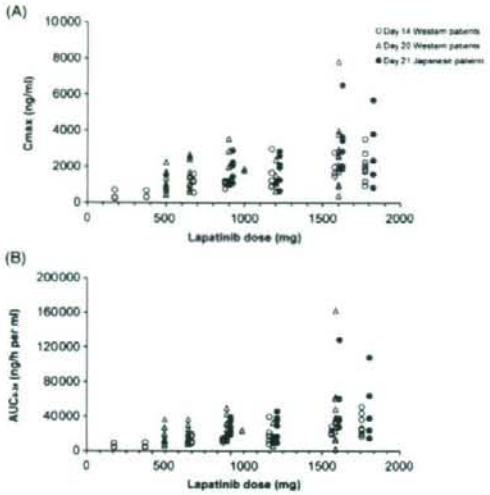

Figure 2. Relation between dose of lapatinib and exposure: comparison of (A) maximum serum concentration (C_{max}) and (B) area under the plasma drug concentration–time curve from 0 to 24 h (AUC_{0-24}) after dosing on Day 21 (our study, Japanese patients) and Days 14 and 20 (US studies, western patients).

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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Phase I/II Study of Docetaxel and S-1 in Patients with Previously Treated Non-small Cell Lung Cancer

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Introduction: The aim of this study was to determine and evaluate the recommended dose of docetaxel in combination with a novel oral 5-fluorouracil analogue S-1 and evaluate the efficacy and safety in patients with previously treated non-small cell lung cancer.

Methods: In phase I, patients with previously treated non-small cell lung cancer were treated with docetaxel (starting dose 40 mg/m^2) intravenously on day 1 and oral administration of S-1 at a fixed dose of 80 mg/m^2 on days 1 to14 every 3 weeks. The recommended dose was the dose level preceding the maximum tolerated dose; once determined, patients were enrolled in phase II.

Results: The recommended dose of docetaxel was 40 mg/m^2 in combination with S-1 80 mg/m^2/d. Of 30 patients enrolled in phase II part, 29 patients were eligible and analyzed. No complete response and 7 (24.1%) partial responses were observed, for an overall response rate of 24.1% (95% confidence interval, 10.3–43.5%). Median overall survival was 11.8 months. The 1-year survival rate was 42%. The grade 3 to 4 hematologic toxicities were neutropenia (34.5%), leukopenia (20.6%), and anemia (10.3%). The grade 3 to 4 nonhematological toxicities included fever 2 (6.9%), diarrhea 1 (3.4%), stomatitis 1 (3.4%), cerebral infarction 1 (3.4%), and pneumonitis 1 (3.4%). There was one treatment-related death due to relapse of drug induced pneumonitis.

Conclusions: This combination chemotherapy is highly active and well tolerated in previously treated patients with non-small cell lung cancer. These results are encouraging and warrant additional investigation.

Key Words: Phase I/II, Non-small cell lung cancer, Second-line chemotherapy, S-1, Docetaxel.

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Lung cancer is the leading cause of tumor-related death worldwide. Non-small cell lung cancer (NSCLC) accounts for approximately 80% of all lung cancers, and by 2002, there were 1.35 million new cases, representing 12.4% of all new cancers.[1] Surgery offers the best chance for cure in stage I and II NSCLC. However, most patients with NSCLC have advanced disease at diagnosis. Chemotherapy is the mainstay of management. The American Society of Clinical Oncology's clinical guidelines recognize that chemotherapy can prolong the survival of advanced NSCLC and is appropriate for those with good PS.[2] The use of doublet regimens has been widely adopted. The principal agents are platinum analogs, taxanes, gemcitabine, irinotecan, and vinorelbine.[3,4] First-line platinum-based chemotherapy is somewhat effective. However, all patients with advanced NSCLC will ultimately progress or relapse. Therefore, second-line chemotherapy is of importance in the clinical management of the patients who had previously received chemotherapy.

Docetaxel has been proven to show antitumor activity against various cancers, including NSCLC.[5–8] This anticancer agent is a mitotic spindle poison that promotes tubulin polymerization and inhibits the depolymerization of microtubules.[9] Docetaxel is one of the standard drugs in second-line chemotherapy. Two recent studies showed improved survival in patients with NSCLC previously treated with platinum in comparison to best supportive care or other drugs.[10,11]

S-1 is a new oral fluorinated pyrimidine. It is a combination drug consisting of a mixture of futraful, 5-chloro-2,4-dihydrozypyridine, and potassium oxonate (Oxo) in a molar ratio of futraful: 5-chloro-2,4-dihydrozypyridine: Oxo = 1: 0.4: 1, based on the biochemical modulation of 5-FU.[12] In phase II studies for advanced NSCLC conducted in Japan, favorable results of S-1 monotherapy or combination therapy have been reported. Kawahara et al. reported that S-1 monotherapy achieved an overall response rate of 22.0% and a median survival time (MST) of 10.2 months.[13] There were no irreversible, severe or unexpected toxicities. Ichinose et al. reported that S-1 plus cisplatin achieved a 47% response rate and a MST of 11 months.[14] Docetaxel and S-1 have shown synergy in human gastric, and breast cancer xenograft models.[15,16] The expression of thymidylate synthase and dihydrouracil dehydrogenase was lower than compared with con-

trol levels. In in vivo experiments using breast cancer xenografts, significant down-regulation of dihydrouracil dehydrogenase activity was observed in tumors treated with S-1, docetaxel and their combination.[16] However, thymidylate synthase activity was not significantly different from control. We hypothesized that the doublet combination chemotherapy using docetaxel and S-1 would have more effect against NSCLC as compared with the monotherapy of docetaxel. The rationale for this combination is that the drugs have different action mechanisms and safety profiles. To improve upon the efficacy of docetaxel alone as second-line treatment, we conducted a phase I/II study of doublet chemotherapy of docetaxel plus S-1.

PATIENTS AND METHODS

Eligibility

Eligible patients were required to have locally advanced or metastatic NSCLC and had failed one or more prior chemotherapy regimens and had at least one measurable lesion. Other main eligibility criteria were as follows: age 20 years or more; Eastern Cooperative Oncology Group performance status (PS) 0 or 1; estimated life expectancy ≥3 months; one or more prior chemotherapy regimens that did not include docetaxel or 5-FU and that was completed >4 weeks before entry; adequate bone marrow, hepatic, renal, and cardiac function [i.e., WBC count ≥4000/μl, absolute neutrophil count ≥2000/μl, platelet count ≥100,000/μl, hemoglobin ≥9.5 g/dl, serum bilirubine level <1.5 mg/dl, aspartate aminotransferase, and alanine aminotransferase within 2.5 times the upper limit of normal (ULN) for the institution, blood urea nitrogen <25 mg/dl, serum creatinine within the ULN, and creatinine clearance ≥60 ml/min]. Exclusion criteria included the presence of other concomitant or metachronous cancers, severe allergy to drugs, simultaneous infectious disease, interstitial pneumonia, or other serious underling medical conditions. The study was approved by the institutional review board of the participating center and all patients provided written informed consent.

Evaluation

All eligible patients who received any part of the treatment were considered assessable for response and toxicity. The complete blood cell counts and blood chemistry studies were measured weekly. The response was assessed based on weekly chest radiograph or computed tomography scan every 4 weeks findings that initially had been used to define tumor extent during the treatment period. The response was evaluated according to the criteria of response evaluation criteria in solid tumors. A complete response (CR) was defined as the complete disappearance of all clinically detectable tumors for at least 4 weeks. A partial response (PR) was defined as an at least 30% decrease in the sum of the longest diameters of the target lesions for more than 4 weeks with no new area of malignant disease. Progressive disease (PD) indicated at least a 20% increase in sum of the longest diameter of the target lesions or a new malignant lesion. Stable disease (SD) was defined as insufficient shrinkage to qualify for PR and insufficient increase to qualify for PD. The best response achieved during the treatment course was reported. Toxicity was graded according to the National Cancer Institute Common Toxicity Criteria version 2.0.[17]

Study Design and Treatment Schedule

This was an open-label multicenter, single-arm phase I/II study in patients with previously treated NSCLC. The objective of the phase I part was to determine the dose-limiting toxicity (DLT), maximum-tolerated dose (MTD), and recommended dose (RD) of docetaxel plus a fixed dose of S-1. In the phase II part, the primary objective was to estimate the overall response rate of this combination at the RD. Secondary objectives were to assess overall survival, 1-year survival rate, adverse events, and progression-free survival (PFS).

In the phase I part of this study, patients received variable doses of docetaxel administered as a 1-hour infusion on day 1 and oral S-1 administered at a fixed dose of 80 mg/m on days 1 to 14 every 3 weeks. S-1 is only available in 20-mg or 25-mg capsules. Therefore, it is easier to plan the dose escalation procedure or a dosage adjustment of docetaxl than S-1. The initial starting dose of docetaxel was 40 mg/m^2 (dose level 1), and step-wise dose increases to 50 (dose level 2) and 60 mg/m^2 (dose level 3) were planned for successive patient cohorts. DLT was determined during the first treatment cycle. At least three patients were enrolled at each dose level: (i) the dose was defined as MTD when two or more of three patients developed DLT; (ii) when one of three patients developed DLT, three other patients were enrolled; (iii) when three or more of six patients developed DLT, the dose was defined as MTD; (iv) when one or two of six patients developed DLT, the dose was increased to the next level.

DLT was defined as follows: grade 4 neutropenia; grade 3 or 4 neutropenia associated with a fever ≥38°C; grade 4 thrombocytopenia; or grade 3 or 4 nonhematological toxicities. A DLT was also reported if 7 days or more omission of S-1, or if the second cycle was delayed until after day 29 because the dosing requirements were not satisfied.

S-1 80 mg/m^2 per day was given orally in 2 divided dose after a meal for 2 weeks, after a drug-free interval of 1 week (one cycle). Three doses of S-1 were selected according to body surface area (BSA). So that they would be approximately equivalent to 80 mg/m^2: BSA<1.25 m^2, 40 mg b.i.d.; BSA 1.25, but <1.5 m^2, 50 mg b.i.d.; and BSA ≥1.5 m^2, 60 mg b.i.d. Docetaxel 40 mg/m^2 was diluted in 500 ml of 0.9% salaine and administered as a 1-hour infusion on the morning of day 1 of each cycle (i.e., every 3 weeks). Dexamethasone 8 mg was infused 1 hour before docetaxel administration. Granulocyte colony-stimulating factor was permitted if a patient developed grade 4 neutropenia; primary prophylaxis was not allowed. Antiemetic (ondansetron) treatment was allowed at the discretion of the treatment physician.

In the phase II part of this study, patients received the RD of docetaxel on day 1 and oral S-1 80 mg/m^2 in accordance with the treatment schedule described above. The treatment was repeated every 21 days for at least two cycles unless there was disease progression, unacceptable toxicity, patient refusal, or the physician's decision to stop treatment.

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S-1 was stopped if there was a leukocyte count of $<2000/\mu l$, neutrophil count of $<1000/\mu l$, platelet count of $<50,000/\mu l$, and a grade 3 or 4 nonhematological toxicity.

The next course of treatment was initiated only when the neutrophil count recovered to $\geq 2000/\mu l$, platelet count to $\geq 100,000/\mu l$, creatinine within the ULN, total bilirubin ≤ 1.5 mg/dl, and the level of aspartate aminotransferase/alanine aminotransferase became <2.5 times the ULN. If patients did not recover from these toxicities within 2 weeks of the last administration of S-1, they were withdrawn from this study. If patients experienced grade 4 neutropenia, fever $\geq 38.0°C$ with grade 3 to 4 neutropenia, grade 3 or more thrombocytopenia, the dose of docetaxel was reduced by 10 mg/m^2 in the subsequent cycle. The dose of S-1 was to be reduced by 20 or 30 mg per day if any grade 3 or 4 nonhematological toxicity was recognized including nausea/vomiting, anorexia, and general fatigue.

Statistical Analysis

Based on the assumption that a response rate of higher than 20% would warrant a further investigation of this combination chemotherapy, and a rate of below 5% would make such an investigation unnecessary, a sample size of 27 patients was required with an alpha error of 0.05 and a beta error of 0.2. Therefore, the accrual of 30 patients was planned for a 2-year period since we considered that several ineligible patients might be identified in the course of the study. PFS was defined as the interval from the start of the treatment to the diagnosis of progression or death from any cause. Overall survival was defined as the interval between when treatment was started and death or the final follow-up visit. Median overall survival and median PFS were estimated by the Kaplan-Meier method.[18] Survival time was recorded at the last confirmation date if the patients were alive.

RESULTS

Between January 2005 and May 2006, 33 patients were enrolled on this study. Nine patients (6 in level 1 and 3 in level 2) were enrolled into the phase I part. Of 30 patients enrolled into the phase II part of the study, one patient did not receive either docetaxel or S-1 because his disease had progressed rapidly. This patient was excluded from all analyses. Twenty-nine patients who were given the RD were evaluated for efficacy and detailed safety profile: these patients consisted of 6 and 23 patients who entered into the study at phase I and II, respectively.

Phase I

The first cohort of 6 patients received docetaxel 40 mg/m^2 plus S-1 80 mg/m^2 (dose level 1). Among these patients, one experienced cerebral infarction (grade 4 CNS cerebrovascular ischemia). No other DLT was observed at dose level 1. At dose level 2 (docetaxel 50 mg/m^2), 2 of the 3 patients developed grade 4 neutropenia which was considered DLT. From these results, the MTD and RD were determined to be level 2 and level 1, respectively.

Phase II

Baseline characteristics of the 29 patients treated at the RD are shown in Table 1. Ages ranged from 48 to 79 years, with a median of 67 years. There were 23 men and 6 women. Nine patients had Eastern Cooperative Oncology Group PS 0, 20 patients had PS 1. Seven patients had clinical stage IIIB disease and 22 had stage IV disease. Histology consisted of adenocarcinoma in 16 patients, squamous cell carcinoma in 10, large-cell carcinoma in 2, and other in one. A single prior chemotherapy regimen had been given in 23 patients, 2 regimen in 4 patients and 3 in 2 patients. Twenty-eight (96.5%) patients had received a platinum-based chemotherapy.

Response and Survival

Of 29 patients assessable for response, none of the patients achieved a CR; 7 (24.1%) achieved a PR with an overall response rate of 24.1% [95% confidence interval (95% CI), 10.3–44.8%]. Thirteen (44.8%) had SD and 7 patients (24.1%) had PD as best response. Two were unevaluable. The tumor control rate (CR + PR+SD) was 68.9% (95% CI, 49.2–84.7%). Among all 29 patients, the median PFS was 3.9 months. As shown in Figure 1, the MST of all patients was 11.8 months, and the 1-year survival rate was 41.8% (95% CI, 21.8–61.8%).

Toxicity of Treatment

Hematological toxicity and nonhematological toxicity were analyzed during treatment and the follow-up period. The major toxicities during the study period are shown in Tables 2 and 3. The grade 3 to 4 hematological toxicities were neutropenia (34.5%), leukopenia (20.6%), and anemia (10.3%). None of the patients developed grade 2 or more thrombocytopenia. The grade 3 to 4 nonhematological toxicities included fever 2 (6.9%), diarrhea 1 (3.4%), stomatitis 1 (3.4%), cerebral infarction 1 (3.4%), and pneumonitis 1 (3.4%). There was one treatment-related death. The patient died 54 days after the first cycle of chemotherapy due to relapse of drug induced pneumonitis.

Treatment Delivery

The median number of cycles administered was 3 (range, 1–8 cycles).

TABLE 1. Patients' Characteristics

No. patients	30
Eligible	29
Male/Female	23/6
Median age, in yr (range)	67 (48–79)
PS 0/1	9/20
ad/sq/la/other	16/10/2/1
Stage IIIb/IV	7/22
No. previous chemo regimens	
1/2/3	23/4/2
RT	13
Operation	3

PS, performance status; Chemo, chemotherapy; RT, radiotherapy.

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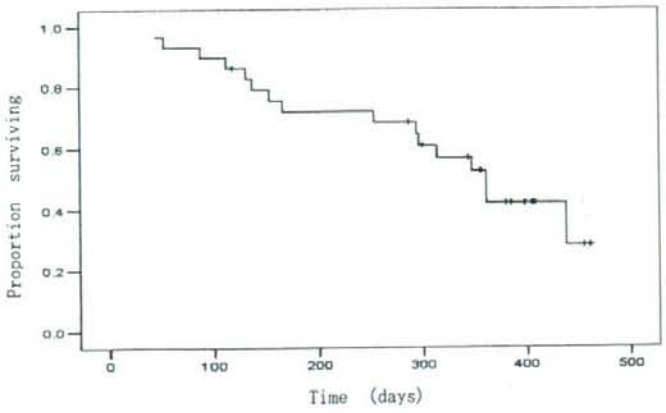

FIGURE 1. Overall survival curve.

TABLE 2. Hematological Toxicity

Grade				
Toxicity	1	2	3	4
Leukopenia	3	8	6	0
Neutropenia	1	5	7	3
Thrombocytopenia	2	0	0	0
Hemoglobin	7	7	3	0

TABLE 3. Nonhematological Toxicity

Grade				
Toxicity	1	2	3	4
Nausea	5	0	0	0
Vomiting	1	2	0	0
Fatigue	1	5	0	0
Infection	0	0	0	0
Fever	3	0	2	0
Diarrhea	4	0	1	0
Ulcer	0	1	0	0
Cerebrovascular ischemia	0	0	0	1
Skin	2	3	0	0
Stomatitis	3	0	1	0
Pneumonitis	1	0	0	1*

*One patient died from relapse of drug induced pneumonitis.

In all, 20 (62.5%) patients received at least 2 cycles of treatment. The reasons for terminating the chemotherapy before the second treatment cycle were disease progression in seven patients and adverse events in two patients. Five patients each required dose reductions of docetaxel or S-1, respectively.

Poststudy Therapy

Eighteen patients received at least one form of antitumoral treatment after disease progression. Thirteen patients received chemotherapy alone, the most frequently prescribed treatment was carboplatin plus gemcitabine. Ten patients received gefitinib.

DISCUSSION

The benefit of second-line chemotherapy has been substantiated by randomized trials using docetaxel, pemetrexed, topotecan, and erlotinib.[10,11,19–21] The response rate was reported to be 6.7 to 10.8% for docetaxel, 9.1% for pemetrexed, 5% for topotecan, and 8.9% for erlotinib. The 1-year survival rate of these reports ranges from 25 to 37%. It is clear that there is an urgent need for more active treatment regimens to patients with relapsed or refractory NSCLC. On the other hand, second-line chemotherapy is a palliative treatment. Therefore, pretreated patients have poorer tolerance to second-line chemotherapy, lower toxicity, and efficacy, which is important when considering the second-line chemotherapy.

To improve the efficacy of second-line chemotherapy, a number of studies have conducted two-drug second-line therapy combinations.[22–25] Georgoulias et al. reported a randomized phase II study that compared single agent irinotecan with a combination of irinotecan plus gemcitabine.[24] Their results failed to demonstrate a statistically significant survival advantage of the combination of irinotecan and gemcitabine over irinotecan alone, although the combination regimen was better in terms of response rate and QOL. A phase III study by Takeda et al. comparing docetaxel alone versus docetaxel plus gemcitabine was terminated early with unexpected incidence of interstitial lung disease and treatment-related deaths due to interstitial lung disease, only in the combination chemotherapy group.[25] Indeed, a comparison of combination chemotherapy versus monotherapy in patients with previously treated NSCLC failed to demonstrate any difference in terms of overall survival. For the moment, single-agent therapy remains the standard option for patients with relapsed or refractory NSCLC.

In the present study, we administered S-1 plus docetaxel to previously treated patients with NSCLC. Seven of the 29 patients (24.1%) achieved a PR as a result. The MST of this regimen was 11.8 months and the 1-year survival rate was 41.8% (Figure 1). The results of the present study are promising, suggesting that the survival of patients treated

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with combination therapy could be improved compared with the survival of those treated with docetaxel alone as a second-line treatment. However, we can not exclude the possibility that the poststudy treatment such as gefitinib or selection bias might also have played a role in prolonging the survival times. Various combination chemotherapy regimens including oral fluoropyrimidine, such as UFUR and capecitabine, have been investigated in NSCLC.[26–28] Kindwall-Keller et al. reported a phase II study of docetaxel and capecitabine in previously treated patients with NSCLC.[27] The response rate was 26% with the MST and 1-year survival rate of 9.1 month and 37%. Chen et al. used UFUR with gemcitabine for 45 patients who failed previous platinum-based chemotherapy.[28] Their patients were treated with 1000 mg/m^2 gemcitabine on days 1 and 8, plus oral UFUR 200 mg/m^2/d from days 1 to 14 of every 3 weeks. They reported that 7 patients (15.6%) had a PR. The MST was 13.2 months.

Our study used 40 mg/m^2 of docetaxel every 3 weeks is lower than that commonly using docetaxel alone at the dose of 75 mg/m^2 as second-line setting in the United States and Europe. By combining docetaxel at 40 mg/m^2 on day 1 with S-1 at 80 mg/m^2/d on days 1 to 14 every 3 weeks, we expected less toxicity, with preserved efficacy. In Japan, docetaxel 60 mg/m^2 every 3 weeks is the commonly used dose. In a phase I study of docetaxel plus S-1, the RD of docetaxel was determined to be 40 mg/m^2 in combination with S-1 80 mg/m^2/d on days 1 to 14. This combination chemotherapy has been evaluated in gastric cancer in Japan.[29–31] The RD of docetaxel was 40 mg/m^2 in combination with S-1 80 mg/m^2/d in the gastric cancer which was the same is our study as a second-line setting. Yamaguchi et al. speculate that the reason for the lower dose of docetaxel may be that the pharmacokinetic parameters (AUC and C_{max}) of 5-FU increase according to the dose of docetaxel.[31]

In our study, the main toxicity was myelosuppression. The most common hematological toxicities were neutropenia and leukopenia. Grade 3 or 4 neutropenia occurred in 34.5% and grade 3 or 4 anemia occurred in 10.3%. In phase III studies of docetaxel 75 mg/m^2 given as a single agent, grade 3 or 4 neutropenia occurred in 40.2 to 67.3% and grade 3 or 4 anemia occurred in 4.3 to 10%.[10,19,20] It seemed that the incidence of grade 3 or 4 neutropenia were lower in our study than in those phase III studies. The majority of nonhematological toxicities were relatively mild. However, grade 4 cerebral infarction and pneumonitis were observed. It is unclear whether this adverse CNS event was related to this combination chemotherapy. This may be due to the hypercoagulability associated with lung cancer. Clotting activation and thromboembolic manifestations are common features in patients with cancer. Therefore, this CNS event might have occurred by chance.[32]

In conclusion, our study indicates that the combination of docetaxel pulse S-1 is an effective and well-tolerated regimen for the treatment of patients with previously treated NSCLC. This regimen seems suitable as a second-line treatment for patients with NSCLC. The response rate and median survival are encouraging and warrant additional investigation.

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Synergistic antitumor effect of S-1 and the epidermal growth factor receptor inhibitor gefitinib in non-small cell lung cancer cell lines: role of gefitinib-induced down-regulation of thymidylate synthase

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Abstract

Somatic mutations in the epidermal growth factor receptor (*EGFR*) gene are associated with the therapeutic response to EGFR tyrosine kinase inhibitors (TKI) in patients with advanced non-small cell lung cancer (NSCLC). The response rate to these drugs remains low, however, in NSCLC patients with wild-type *EGFR* alleles. Combination therapies with EGFR-TKIs and cytotoxic agents are considered a therapeutic option for patients with NSCLC expressing wild-type *EGFR*. We investigated the antiproliferative effect of the combination of the oral fluorouracil S-1 and the EGFR-TKI gefitinib in NSCLC cells of differing *EGFR* status. The combination of 5-fluorouracil and gefitinib showed a synergistic antiproliferative effect *in vitro* in all NSCLC cell lines tested. Combination chemotherapy with S-1 and gefitinib *in vivo* also had a synergistic antitumor effect on NSCLC xenografts regardless of the absence or presence of *EGFR* mutations. Gefitinib inhibited the expression of the transcription factor E2F-1, resulting in the down-regulation of thymidylate synthase at the mRNA and protein levels. These observations suggest that gefitinib-induced down-regulation of thymidylate synthase is responsible, at least in part, for the synergistic antitumor effect of combined treatment with S-1 and gefitinib and provide a basis for clinical evaluation of combination chemotherapy with S-1 and EGFR-TKIs in patients with solid tumors. [Mol Cancer Ther 2008;7(3):599–606]

Introduction

Targeted therapy in the treatment of cancer has made substantial progress over the last few years. The ErbB family of receptor tyrosine kinases includes the epidermal growth factor receptor (EGFR; ErbB1), ErbB2 (HER2/*neu*), ErbB3, and ErbB4 and is important for normal development as a result of its roles in cell proliferation and differentiation (1–3). Aberrant expression of EGFR has been detected in a wide range of human epithelial malignancies, including non-small cell lung cancer (NSCLC), and is correlated with poor prognosis and reduced survival time (4, 5). Agents that specifically target EGFR are therefore under development as anticancer drugs. Indeed, two inhibitors of the tyrosine kinase activity of EGFR (EGFR-TKI), gefitinib and erlotinib, both of which compete with ATP for binding to the catalytic pocket of the receptor, have been extensively studied in individuals with NSCLC (6–9). Somatic mutations in the region of *EGFR* that encodes the tyrosine kinase domain have been associated with tumor responsiveness to EGFR-TKIs in a subset of NSCLC patients (10–17). In contrast, achievement of a clinical benefit of these drugs in NSCLC patients who express wild-type *EGFR* has been problematic.

S-1 (Taiho Pharmaceutical) is an oral anticancer agent composed of tegafur, 5-chloro-2,4-dihydroxypyridine (CDHP), and potassium oxonate in a molar ratio of 1:0.4:1 (18). Tegafur is a prodrug that generates 5-fluorouracil (5-FU) in blood largely as a result of its metabolism by cytochrome *P*450 in the liver. CDHP increases the plasma concentration of 5-FU through competitive inhibition of dihydropyrimidine dehydrogenase (DPD), which catalyzes 5-FU catabolism (19). Oxonate reduces the gastrointestinal toxicity of 5-FU (20). A response rate of 22% and a median survival time of 10.2 months were obtained in a clinical trial of S-1 in patients with advanced NSCLC not subjected previously to chemotherapy (21). Few severe gastrointestinal or hematologic adverse events were reported. Moreover, a phase II trial of S-1 plus cisplatin in NSCLC patients revealed a 47% response rate and an acceptable safety profile (22).

Based on this background, we examined the anticancer effect of the combination of S-1 and gefitinib in NSCLC cell lines of differing *EGFR* status. We found that the combination of S-1 (or 5-FU) and gefitinib exhibited a marked and synergistic antiproliferative effect both *in vivo*

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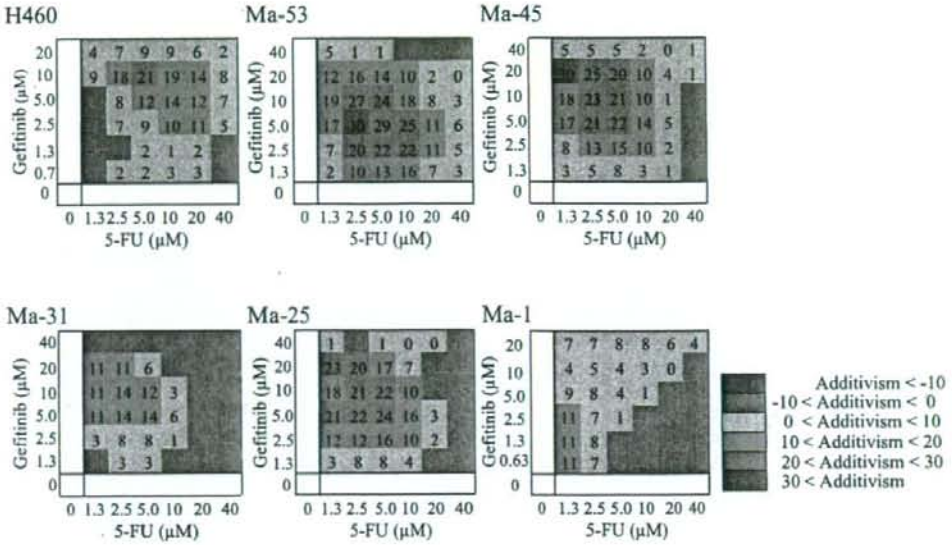

Figure 1. Inhibition of NSCLC cell growth by the combination of 5-FU and gefitinib *in vitro*. Cells with wild-type (H460, Ma-53, Ma-45, Ma-31, and Ma-25) or mutant (Ma-1) EGFR alleles were exposed for 72 h to 5-FU and gefitinib at the indicated concentrations, after which cell viability was measured with a colorimetric assay. The observed excess inhibition (%) relative to that predicted by the Bliss additivism model is shown color-coded in a drug concentration matrix for each cell line. Yellow, orange, pink, and red, synergy; light and dark blue, antagonism. Mean of triplicates from a representative experiment.

and *in vitro* in cells regardless of the absence or presence of *EGFR* mutations. Furthermore, we assessed the effects of gefitinib on the expression of enzymes that function in 5-FU metabolism, including thymidylate synthase (TS), DPD, and orotate phosphoribosyltransferase (OPRT), to gain insight into the mechanism underlying the synergistic effect of combination therapy with S-1 and gefitinib.

Materials and Methods

Cell Lines and Reagents

The human NSCLC cell lines NCI-H460 (H460), Ma-1, Ma-25, Ma-31, Ma-45, and Ma-53 were obtained as described previously (23). MiaPaca-2 cells were obtained from Japan Health Sciences Foundation. These cell lines were cultured under a humidified atmosphere of 5% CO_2 at 37°C in RPMI 1640 (Sigma) supplemented with 10% fetal bovine serum. Gefitinib was provided by AstraZeneca. S-1 and CDHP were provided by Taiho Pharmaceutical. 5-FU was obtained from Wako.

Growth Inhibition Assay *In vitro*

Cells (2.0×10^3) were plated in 96-well flat-bottomed plates and cultured for 24 h before the addition of various concentrations of 5-FU and gefitinib and incubation for an additional 72 h. Cell Counting Kit-8 solution (Dojindo) was then added to each well, and the cells were incubated for 3 h at 37°C before measurement of absorbance at 450 nm. Absorbance values were expressed as a percentage of that for untreated cells, and the concentration of 5-FU or gefitinib resulting in 50% growth inhibition (IC_{50}) was calculated. The effect of combining 5-FU and gefitinib was classified as additive, synergistic, or antagonistic with the Bliss additivism model (24–26). A theoretical curve was calculated for combined inhibition with the equation: $E_{bliss} = E_A + E_B - (E_A \times E_B)$, where E_A and E_B are the fractional inhibitory effects of drug A alone and drug B alone at specific concentrations. E_{bliss} is then the fractional inhibition that would be expected if the effect of the combination of the two drugs was exactly additive. In this study, the Bliss variable is expressed as percentage decrease in cell growth above what would be expected for the combination. Bliss = 0 indicates that the effect of the combination is additive; Bliss > 0 is indicative of synergy; and Bliss < 0 indicates antagonism.

Animals

Male athymic nude mice were exposed to a 12-h light, 12-h dark cycle and provided with food and water *ad libitum* in a barrier facility. All experiments were done in compliance with the regulations of the Animal Experimentation Committee of Taiho Pharmaceutical.

Growth Inhibition Assay *In vivo*

Cubic fragments of tumor tissue (~2 × 2 × 2 mm) were implanted s.c. into the axilla of 5- to 6-week-old male athymic nude mice. Treatment was initiated when tumors in each group achieved an average volume of 100 to 150 mm^3. Treatment groups consisted of control, S-1 alone, gefitinib alone, and the combination of S-1 and gefitinib. Each treatment group contained seven mice. S-1 (10 mg/kg body mass) and gefitinib (50 or 3 mg/kg) were administered by oral gavage once a day for 14 days; control animals