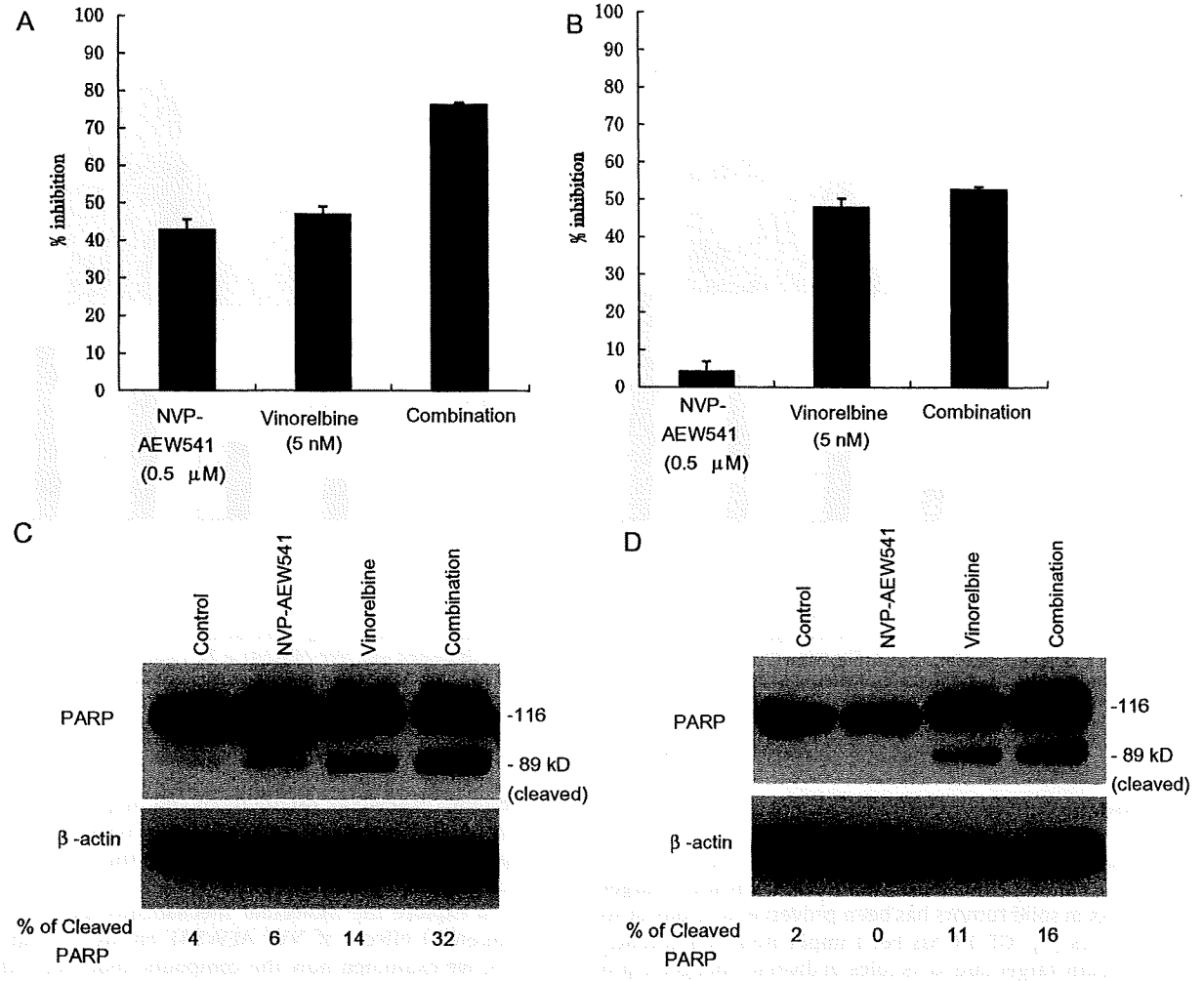

Fig. 5. Effect of NVP-AEW541 on vinorelbine-induced apoptosis in MCF-7. MCF-7 (A and C) and T47D (B and D) cell lines were treated with 0.5 μM of NVP-AEW541, 5 nM of vinorelbine, and their combination. (A and B) Viable cells were quantified by the MTS assay and are expressed as a percentage relative to untreated controls (*y*-axis). Each data point represents the mean value and standard deviation of 6–12 replicate wells. (C and D) Western blot for PARP with MCF-7 and T47D grown in the absence or presence of 0.5 μM of NVP-AEW541, 5 nM of vinorelbine, and their combination. The blot was stripped and reprobed for β-actin as a loading control. Percentage of the cleaved form (89-kDa) was quantified and is shown at the bottom of the blots.

and was inhibited by NVP-AEW541 (Fig. 7A). This inhibition coincided with a decrease in phosphorylation of Akt (Fig. 7A). In contrast, the level of phospho-Akt in T47D cells was much higher than in MCF-7 and was decreased only slightly by NVP-AEW541 (Fig. 7A). Phosphorylation of ERK1/2 was not affected NVP-AEW541 in either cell line, nor was the total amount of any of these signaling proteins (Fig. 7A). To test the effects of NVP-AEW541 on ligand-dependent cell signaling, we serum-starved MCF-7 and T47D cells overnight and stimulated them with IGF-1 for 15 min with or without 2 h pre-exposure to 1 μM NVP-AEW541, then analyzed lysates of the cells by Western blotting (Fig. 7B). In both cell lines, phosphorylation of IGF-1R dramatically increased with the IGF-1 stimulation, and was significantly inhibited by NVP-AEW541 pre-treatment. Interestingly, in MCF-7 cells phosphorylation of IRS-1 was observed even without ligand-stimulation, and was increased by addition of IGF-1 (Fig. 7B). NVP-AEW541 inhibited the IGF-1-dependent phosphorylation of IRS-1 and phosphorylation of Akt in MCF-7 cells (Fig. 7B). In T47D, in contrast, IRS-1 phosphorylation was barely detectable and did not change with addition of IGF-1 or NVP-AEW541 (Fig. 7B). Accordingly, the level of Akt phosphorylation changed only slightly (Fig. 7B). On the other hand, while phosphorylation of ERK1/2 was increased by IGF-1 and inhibited by NVP-AEW541 in T47D cells, it was unaffected in MCF-7 (Fig. 7B).

Taken together, these results indicate that inhibition of cells' proliferation, cell-cycle progression, survival, and motility by NVP-AEW541 does not correlate with inhibition of IGF-1R phosphorylation, but rather with decreased phosphorylation of Akt. The role of the ERK1/2 pathways appeared relatively small when compared to the PI3 K pathways, at least for the oncogenic properties we tested.

3.8. NVP-AEW541 disrupts IRS-1/PI3 K complex

In activating PI3 K, IGF-1R is known to utilize IRS-1 as a scaffold protein [10]. Because inhibition of the PI3 K pathway appeared to play an important role in the mechanism of action of NVP-AEW541, we next examined the effect of the compound on the interaction between IRS-1 and p85. MCF-7 and T47D cells were treated with or without 1 μM NVP-AEW541 for 24 h, and cellular protein extracts were immunoprecipitated with p85 antibody and analyzed by immunoblotting with IRS-1 and p85 antibodies. As shown in Fig. 7C, IRS-1 and p85 are physically associated in MCF-7 cells and this association is disrupted by NVP-AEW541. In contrast, the two proteins are not associated in T47D cells. These findings are consistent with our Western blot analysis showing that NVP-AEW541 suppresses phosphorylation of Akt in MCF-7 but not in T47D (Fig. 7A and B).

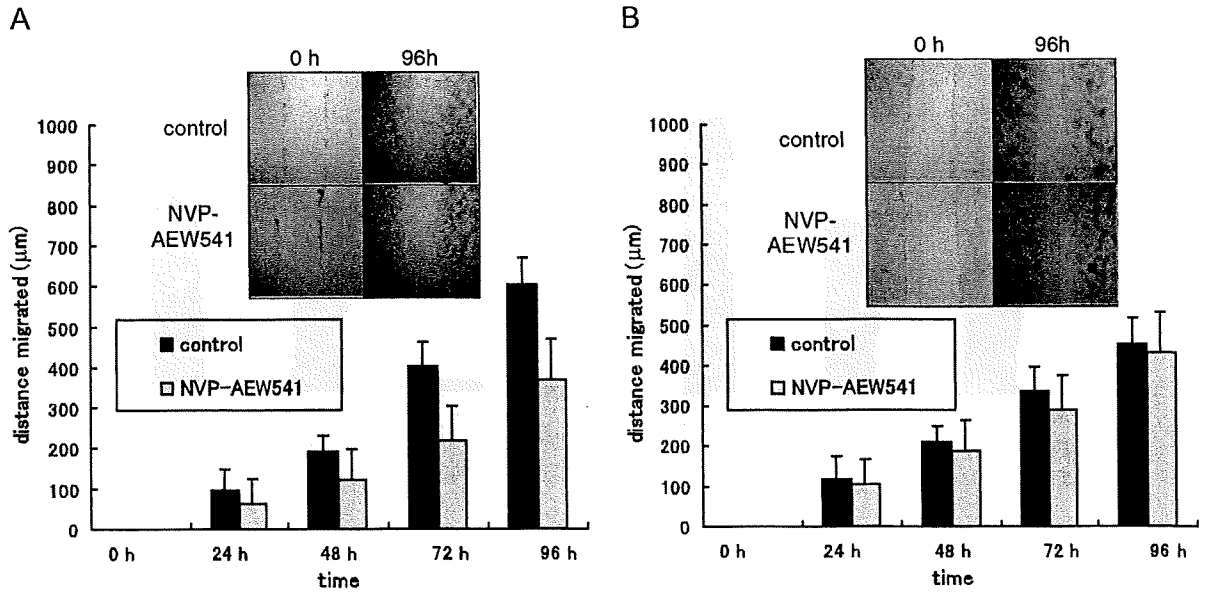

Fig. 6. Inhibitory effect of NVP-AEW541 on migration of MCF-7. MCF-7 and T47D cell lines were grown to confluence and treated with 1 μM NVP-AEW541 before an *in vitro* scratch assay. The scratched region was photographed at the indicated times and the migration distance was measured at three fixed positions (top, middle, and bottom). (A) The migration is significantly slower in MCF-7 cells treated with NVP-AEW541 at 72 h and 96 h ($P < 0.05$). Each data point represents the mean and standard deviation of 6–12 replicate wells. (B) In T47D cell line, NVP-AEW541 produces no significant change in the rate of migration.

4. Discussion

Over the last decade, a number of molecularly-targeted agents have entered clinical use, and the efficacy of targeting RTKs in solid tumors has been proven efficacious in solid tumors [7]. IGF-1R has been implicated as a potential therapeutic target due to its roles in diverse oncogenic processes including cell proliferation, survival, motility, and metastasis [10]. In the present study, we examined *in vitro* effects of selectively inhibiting IGF-1R with NVP-AEW541.

We first found that even though all 16 of the breast cancer cell lines tested express IGF-1R, and many of them show baseline phosphorylation of this RTK, one cell line, MCF-7, was much more sensitive to NVP-AEW541 than the others in assays of cell growth. Interestingly, we found that of the 16 cell lines, only MCF-7 has high expression and phosphorylation of both IGF-1R and its substrate IRS-1 (Fig. 1 and Table 1). We hypothesized that concurrent expression and phosphorylation of both proteins could play a role in the mechanism of action of NVP-AEW541. To test this hypothesis we chose to compare MCF-7 and T47D cells, because these lines had identical expression and phosphorylation levels of IGF-1R, and almost identical profiles of other phospho-RTKs (Fig. 2), but differ in that MCF-7 cells have much higher expression and phosphorylation of IRS-1 (Fig. 1 and Table 1).

We found that in MCF-7 but not in T47D, 1 μM NVP-AEW541 causes G1–S cell-cycle arrest, consistent with the higher growth inhibition observed in MCF-7. We also found that in MCF-7 but not in T47D, NVP-AEW541 enhanced the cytotoxicity of vinorelbine and paclitaxel. Furthermore, NVP-AEW541 retarded cell migration in MCF-7 but not T47D. These observations are consistent with the reported contribution of IGF-1R signaling to the cellular processes measured in these assays.

To explore the molecular mechanisms underlying the differential effects of NVP-AEW541 on MCF-7 and T47D cells, we examined how the compound modified different cell signaling pathways. In MCF-7 but not T47D cells, 1 μM NVP-AEW541 significantly inhibited Akt phosphorylation, both in the presence of 10% serum and after serum starvation followed by IGF-1 treatment (Fig. 7). In contrast, NVP-AEW541 did not affect the ERK pathway, another well-characterized proliferation and survival pathway, in cells of either line grown in 10% serum. Furthermore, we demonstrated that NVP-AEW541 dissociated IRS-1/PI3 K in MCF-7 but not in T47D cells. These findings suggest that cellular sensitivity to NVP-AEW541 is determined by whether the signaling axis of IGF-1R–IRS-1–PI3 K is functional. The presence of the axis may be indicated by high expression of both IGF-1R and IRS-1, as discussed below. Alternatively, dissociation of IGF-1R and IRS-1 may be naturally present in some cancer cells, because in T47D stimulation with IGF-1 caused only a small increase in phosphorylation of IRS-1 (Fig. 7B). The PI3 K/Akt pathway is known to be multifunctional, mediating important oncogenic processes such as cell proliferation, cell survival, and cell motility [27]. This is again consistent with the present findings that NVP-AEW541 inhibited not only cell growth but other oncogenic properties in MCF-7.

Considering therapeutic implications, our results suggest that measuring IGF-1R expression alone provides insufficient information to select tumors sensitive to

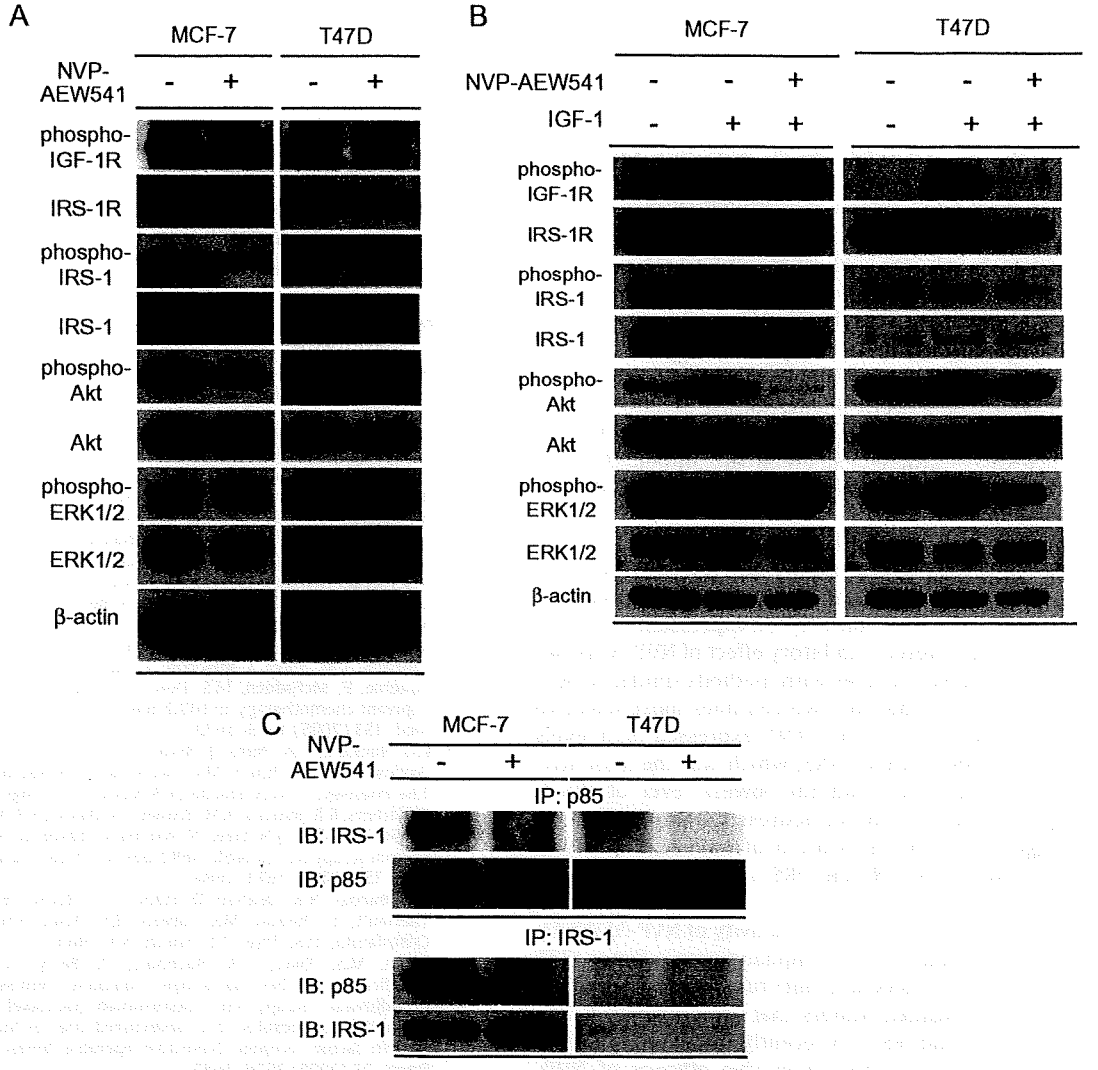

Fig. 7. Effects of NVP-AEW541 on phosphorylation of IGF-1R and downstream signaling molecules. (A) MCF-7 and T47D cell lines were treated with 1 μM NVP-AEW541 for 24 h and the levels of phosphorylated IGF-1R, Akt, and ERK1/2 were assessed using phospho-specific antibodies for each protein. The blot was stripped and reprobed with an antibody detecting the total form of the protein, and again with antibody for β-actin as loading control. (B) Effects of IGF-1 and NVP-AEW541 on phosphorylation of IGF-1R and downstream Akt and ERK1/2. MCF-7 and T47D cells were serum starved overnight and then grown in the presence or absence of 1 μM NVP-AEW541 for 2 h followed by IGF-1 (50 ng/ml) stimulation for 15 min. Western blots are shown for phospho- and total IGF-1R, Akt, and ERK1/2. (C) MCF-7 and T47D cell lines were grown in the presence or absence of NVP-AEW541 for 24 h. Protein extracts (500 μg) were immunoprecipitated with an anti-p85 antibody and subjected to immunoblot assays with anti-IRS-1 and anti-p85 antibodies. In MCF-7, the association of IRS-1 and p85 is significantly diminished in the presence of NVP-AEW541.

NVP-AEW541, consistent with a previous study by Scotlandi et al. using musculoskeletal cancer cell lines [21]. Our results rather suggest that the presence of high IRS-1 expression beside IGF-1R expression indicates the presence of a signaling axis from IGF-1R to PI3 K, and would thus be informative for selecting NVP-AEW541-sensitive tumors. This concept is supported by a recent study by Byron, et al. [28]. In the study, they showed that in T47D-YA breast cancer cells expressing active IGF-IR but lacking IRS-1, IGF-1 did not stimulate cell proliferation but did when cDNA constructs encoding human IRS-1 were stably transfected into the cells [28]. They also showed that MCF-7 cells with IRS-1 knocked down by means of interfering RNA (siRNA) exhibited diminished IGF-1-stimulated cell growth compared to parental cells [28]. Despite the high frequency of IRS-1 expression in breast cancer specimens reported in a previous study [29], 69.7%, the definition and the frequency of over-expression of IRS-1 remains unclear. If the level of IRS-1 MCF-7 shows should be regarded as over-expression, it may be infrequent in breast cancer. It also remains unknown how frequent IGF-1R and IRS-1 simultaneously over-express in breast cancer. However, because a previous study showed that higher levels of IRS-1 predicted worse disease-free survival after curative surgery in a subset of breast cancer patients [19], NVP-AEW541 might be efficacious against relatively aggressive tumors.

In T47D cells, phosphorylation of Akt is high even after serum starvation (Fig. 7B), suggesting the presence of a

mechanism causing constitutive activity of the PI3 K pathway. One possible candidate is an activating mutation of p110 (H1047R in exon 20) in T47D [30]. Interestingly, however, MCF-7 is also reported to have an activating mutation in p110, though at a different site (E545 K in exon 9) [30]. We confirmed the presence of these mutations in each cell line for ourselves (data not shown). Both mutations have been shown to cause equal elevation of PI3 K catalytic activity when expressed in a normal mammary epithelial cell line [31]. However, our data showed that phosphorylation of Akt in the absence of serum is much lower in MCF-7 than T47D cells, and is boosted by addition of exogenous IGF-1 (Fig. 7B), which suggests that these two mutations may have different effects in cancer cell lines in which they occur endogenously. In addition, it is of therapeutic importance to know that cells with mutant PI3 K, like MCF-7, still depend on up-stream signals in terms of PI3 K activation, and therefore can be sensitive to anti-RTK drugs.

Our study has some limitations. First, the use of only one cell line with high sensitivity to NVP-AEW541 precludes generalization of the results. In a recently published study by Guerreiro, et al., they tested expression of IGF-1R and IRS-1 and the growth inhibitory effect of NVP-AEW541 for 9 neuroblastoma cell lines with methods similar to ours [32]. They observed that the two cell lines most sensitive to NVP-AEW541, SHSY5Y and LAN1, expressed high levels of IGF-1R and IRS-1, while LAN5, which was the least sensitive to NVP-AEW541, had the lowest level of IRS-1. Although the authors did not address the issue, their results suggest that the association of NVP-AEW541 with co-expression of IGF-1R and IRS-1 may be a general phenomenon.

Second, we did not test *in vivo* activity of NVP-AEW541. Because IGF-1 could act as a lymphangiogenic factor [33], our result does not preclude anti-tumor effects of NVP-AEW541 *in vivo* against tumors that are unlike MCF-7 in terms of IGF-1R and IRS-1. A recently published study by Tanno and colleagues showed *in vivo* efficacy of NVP-AEW541 for neuroblastoma cell lines, but they were shown to be sensitive *in vitro* as well [34]. Therefore, it remains to be addressed, and is a focus of our ongoing study, whether NVP-AEW541 can work *in vivo* especially for cancer cells that are insensitive *in vitro*.

In summary, our study supports the possibility of targeting IGF-1R in breast cancer. Inhibiting IGF-1R and consequently the PI3 K/Akt pathway is therapeutically attractive as it may not only inhibit cell growth but also enhance the effect of chemotherapeutic agents and reduce the ability of cells to migrate to other sites. However, *in vitro* effects of IGF-1R receptor inhibition in breast cancer cells appeared to be limited to those that express both IGF-1R and IRS-1 at high levels. Although the validity of the findings in our study should be evaluated in clinical trials, they could potentially lead to individualized use of NVP-AEW541 in breast cancer.

Conflict of interest statement

None declared.

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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.

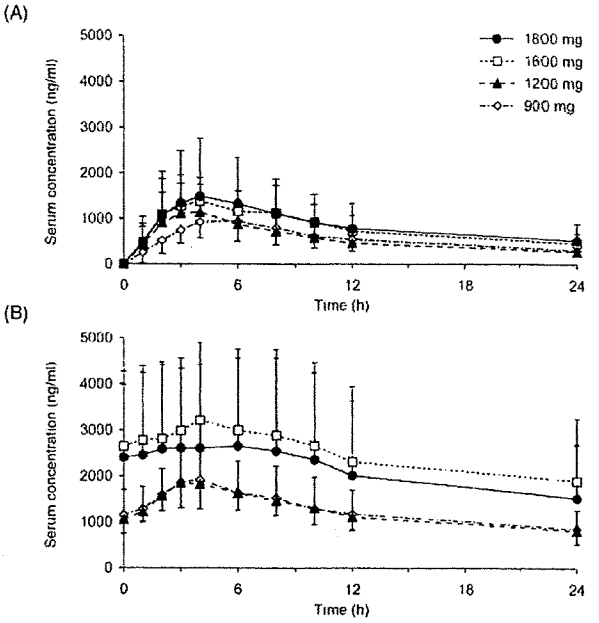

Figure 1. Serum concentrations of lapatinib at each dose level as detected on (A) Day 1 and (B) Day 21.

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

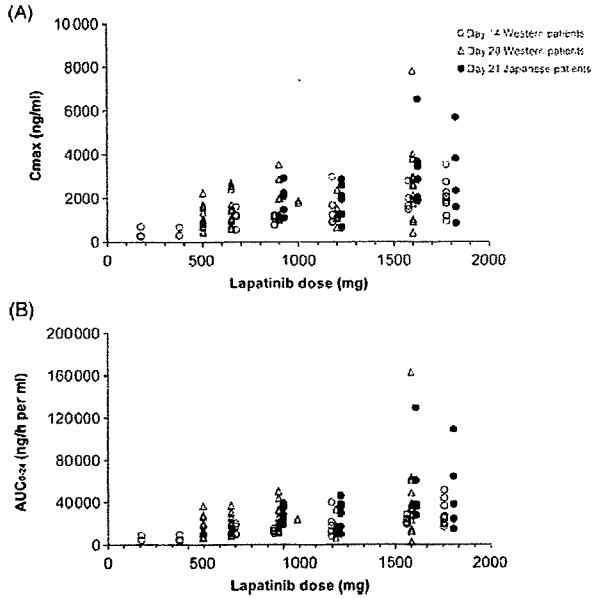

Figure 2. Relation between dose of lapatinib and exposure: comparison of (A) maximum serum concentration (C_{max}) and (B) area under the plasma drug concentration–time curve from 0 to 24 h (AUC_{0-24}) after dosing on Day 21 (our study, Japanese patients) and Days 14 and 20 (US studies, western patients).

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 and pharmacokinetic study of sorafenib, an oral multikinase inhibitor, in Japanese patients with advanced refractory solid tumors

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Sorafenib is a novel oral multikinase inhibitor that targets Raf serine/threonine and receptor tyrosine kinases, and inhibits tumor cell proliferation and angiogenesis. We have conducted a phase I study of sorafenib to determine the safety, tolerability, pharmacokinetics, and potential efficacy of this agent in 31 Japanese patients with advanced refractory solid tumors. Sorafenib (100–600 mg) was given as a single dose followed by a 7-day wash-out period, and then administrated twice daily (bid). The most frequent drug-related adverse events were rash/desquamation (61%), hand–foot skin reactions (39%), diarrhea (36%), and elevations of serum lipase (36%) and amylase (26%) levels. Dose-limiting toxicities (DLTs) were grade 3 diarrhea at 200 mg bid and grade 3 fatigue at 600 mg bid. Grade 3 and 4 pancreatic enzyme elevations were observed at 200–600 mg bid, but they were not deemed dose-limiting because they were asymptomatic and were not associated with pancreatitis or chronic damage to the pancreas. The AUC and C_{max} of sorafenib increased linearly with dose up to 400 mg bid. Partial responses were observed in one of 10 patients with non-small cell lung cancer and one of three patients with renal cell carcinoma. In conclusion, sorafenib 400 mg bid was well tolerated in Japanese patients with advanced refractory solid tumors. The recommended dose for future clinical trials is 400 mg bid. (*Cancer Sci* 2008; 99: 1492–1498)

Recent research on the molecular mechanisms controlling tumor cell proliferation, invasion, and metastasis has identified several novel targets for cancer therapeutics. The mitogen-activated protein kinase (MAPK) signaling pathways, which mediate transduction of extracellular signals to the nucleus via a cascade of phosphorylation events through Ras/Raf/MEK/ERK, are often dysregulated in human tumors. Dominant negative mutants of Raf or ERK inhibit both the primary and metastatic growth of human tumor xenografts *in vivo*. Thus, activation of Raf kinase is considered to be an important mechanism by which human cancer develops. Therefore, the critical components of MAPK signaling pathways, including Raf kinase, represent potential targets for anticancer treatment.[1–4]

Tumor angiogenesis, the proliferation of a vascular network to supply tumors with nutrients and oxygen, is necessary for tumors to maintain growth and to spread. It is supported by angiogenic factors such as vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF). VEGF and PDGF bind the VEGF receptor (VEGFR) on endothelial cells and the PDGF receptor (PDGFR) on smooth muscle cells, which are both receptor tyrosine kinases, respectively. Thus, the receptors themselves and their signaling pathways are also potential therapeutic targets for cancer.[5,6]

Sorafenib (BAY 43-9006) is an orally available small molecule that displays inhibitory activity against multiple kinases including c-Raf-1 and B-Raf. Inhibition of Raf activity is followed by interference with the activation of ERK, thereby inhibiting cell proliferation, differentiation, and transformation.[7,8] In addition, sorafenib inhibits receptor tyrosine kinases including VEGFR-2 and PDGFR, thereby inhibiting angiogenesis. Inhibition of both tumor cell proliferation and angiogenesis is considered to contribute to the potent antitumor activity of sorafenib. In studies of various human tumors, sorafenib exhibited a dose-dependent inhibition of tumor growth associated with apoptosis in xenograft models.[7,8]

Various types of clinical development programs for sorafenib are now on-going worldwide. In the phase III Treatment Approaches in Renal Cancer Global Evaluation Trial (TARGET), sorafenib significantly prolonged progression-free survival as well as overall survival in patients with advanced renal cell cancer.[9] Sorafenib has recently been approved for advanced renal cell carcinoma and hepatocellular carcinoma in the USA, Europe, and other countries.

The phase I study reported here was planned to determine the safety, dose-limiting toxicities (DLTs), maximum-tolerated dose (MTD), and pharmacokinetics of sorafenib in Japanese patients with refractory advanced solid tumors. Pharmacodynamics was also studied using flow cytometric analysis of ERK-phosphorylation in patients' peripheral blood mononuclear cells (PBMCs), as well as plasma adrenomedullin levels. Furthermore, disease activity was evaluated by fluorodeoxyglucose-positron emission tomography (FDG-PET).

Materials and Methods

Patient selection. Study eligibility criteria included histologically or cytologically confirmed advanced solid cancer, which was refractory to standard therapy or for which no effective therapy was available, patient age ≥ 20 years, Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1, estimated life expectancy ≥ 12 weeks, and adequate organ function. Main exclusion criteria were as follows: chronic heart failure (New York Heart Association Grade III or IV), active cardiac diseases, history of HIV infection or chronic hepatitis B or C, active infections, tumor involving the central nervous system, history of seizure, concurrent malignancy, other anticancer therapy within 4 weeks (6 weeks for mitomycin C or nitrosourea, 2 weeks for hormonal therapy, and 3 weeks for radiotherapy), and surgery within 4 weeks prior to this study. Patients treated with CYP3A4 inhibitors or inducers were also excluded because of possible drug interactions with sorafenib and confounding effects

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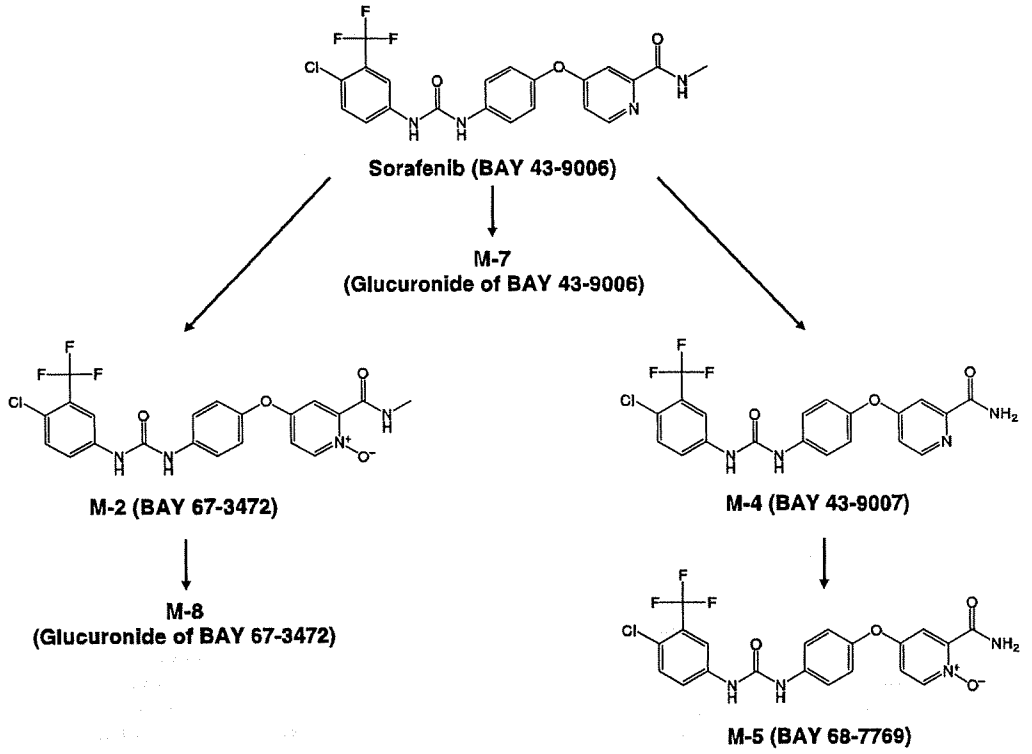

Fig. 1. Metabolism map of sorafenib and its metabolites.

on the pharmacokinetics results. The study was approved by the Institutional Review Board of the National Cancer Center and all patients gave written informed consent before entry onto the study.

Study design. A single dose of sorafenib was given orally, followed by a 7-day wash-out, and then administration of sorafenib continued twice daily until the occurrence of unacceptable toxicity, withdrawn consent, disease progression, or death.

In this study, the initial dose was 100 mg, which was based on observations in phase I studies performed in foreign countries as well as on preclinical studies. In both dogs and rats, exposures to between 53.5 and 67.1 mg h/L was associated with moderate toxicity. Assuming that oral bioavailability is similar in humans, a single 100 mg dose of sorafenib would be expected to yield a systemic exposure of 5.8 mg h/L. Therefore, 100 mg sorafenib was considered to be a safe starting dose for this phase I study, thereafter escalated to 200, 400, and 600 mg bid.

For each dose level, a cohort of three patients was treated. In the absence of observed DLTs during the first 4 weeks of continuous administration bid, a further cohort of three patients was enrolled to the next higher dose. If one of the first three patients experienced DLTs, three additional patients were treated at that same dose level. The dose was then escalated when no DLTs was observed in the three additional patients.

Definition of dose-limiting toxicity. Toxicities were evaluated according to the National Cancer Institute Common Toxicity Criteria (NCI-CTC) version 2.0, with DLTs being defined as grade 3 or 4 non-hematological toxicity (except anorexia and manageable nausea and vomiting), grade 4 neutropenia lasting for ≥7 days, febrile neutropenia, or thrombocytopenia <25 000/mm^3.

Grade 4 elevations of pancreatic enzymes were observed in 200 mg bid cohorts, but ultrasound investigation, magnetic resonance imaging, and computed tomography did not show any evidence of pancreas damage or pancreatitis. Therefore, after the safety of 200 mg bid was confirmed, the definition of DLT was amended to exclude clinically insignificant elevations of pancreatic enzymes and the definition of DLT for serum pancreatic enzymes was amended accordingly. DLTs were deemed dose-limiting only when they were grade 4 for >4 days, associated with clinical/imaging findings of pancreatitis, or considered to be life-threatening or result in chronic damage to the pancreas.

Patient evaluation. Physical examination and hematological/biochemical laboratory evaluation were performed weekly for the first 4 weeks of continuous dosing and every 2 weeks thereafter. Laboratory evaluation was also performed on day 4 of the continuous dosing. Tumor measurements were performed at the baseline, and repeated every 8 weeks according to the Response Evaluation Criteria in Solid Tumors (RECIST).[10] Tumor responses were classified as complete response (CR), partial response (PR), stable disease (SD), and progressive disease (PD).

Pharmacokinetics. For the measurement of plasma concentrations of sorafenib and its metabolites, blood samples (5 mL aliquots) were drawn prior to drug administration, as well as 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 12, 24, 36, 48, 72, 96, and 120 h after the single dose administration. For the continuous dosing period, blood was sampled prior to the first dosing on days 1, 4, 7, 10, 14, 21, and 28, along with 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, and 12 h after the first dose on day 14 at 100, 200, 400, and 600 mg bid. The same full sampling was performed on day 28 at 100 and 200 mg bid, while blood was sampled prior to and 12 h after the morning administration at 400 and 600 mg bid. Urine voided up to 48 h after the single administration was collected.

Concentrations of sorafenib and its metabolites in plasma and urine were determined at Bayer HealthCare (Berlin, Germany) using high performance liquid chromatography-tandem mass spectrometry (HPLC-MS-MS) methods.[11] The method was validated within a working range of 0.0100–12.0 mg/L (sorafenib) and 0.0100–2.5 mg/L (metabolite M2; M4; M5; Fig. 1). Mean interassay precision and accuracy for sorafenib quantification ranged from 0.4% to 4.9% and from 91.2% to 96.5%, respectively. Plasma pharmacokinetic parameters, area under the curve

Table 1. Patient characteristics

Number of patients (female/male)	31 (10/21)
Median age (range)	63 (32–72)
ECOG performance status	
0	8
1	23
Cancer type	
Non-small cell lung	10
Colorectal	6
Renal	3
Gastric	2
Others	10
Prior therapy	
Chemotherapy	30
Radiotherapy	11
Surgery	29

EOCG, Eastern Cooperative Oncology Group.

Table 2. Incidence of drug-related adverse events by worst grade

Adverse event	All grades *n* (%)	Grade 3 *n* (%)	Grede 4 *n* (%)
Hypertension	4 (13%)	1 (3.2%)	0
Fatigue	3 (10%)	1 (3.2%)	0
Fever	3 (10%)	0	0
Alopecia	8 (26%)	0	0
Dry skin	7 (23%)	0	0
Hand–foot skin reaction	12 (39%)	0	–
Rash/desquamation	19 (61%)	0	–
Pruritus	5 (16%)	0	0
Anorexia	8 (26%)	0	0
Diarrhea	11 (36%)	1 (3.2%)	0
Nausea	3 (10%)	0	0
Vomiting	3 (10%)	0	0
Lipase	11 (36%)	2 (6.5%)	5 (16%)
Amylase	8 (26%)	2 (6.5%)	1 (3.2%)
Alkaline phosphatase (ALP)	3 (10%)	1 (3.2%)	0
Alanine amino transferase (ALT)	3 (10%)	1 (3.2%)	1 (3.2%)
Aspartic aminotransferase (AST)	3 (10%)	1 (3.2%)	2 (6.5%)
Abdominal pain	5 (16%)	0	0
Leukocytopenia	4 (13%)	4 (13%)	0

(AUC), maximum concentration (C_{max}), and elimination half-life ($t_{1/2}$) for sorafenib were calculated by non-compartment analysis using the KINCALC program (Bayer HealthCare).

Pharmacodynamics. As a specific marker for the Ras signaling pathway, phosphorylated ERK (pERK) in peripheral blood mononuclear cells (PBMC) was quantified. Peripheral blood samples with EDTA anticoagulant were taken at the baseline and on day 28 of the continuous treatments. PBMCs were prepared from blood, stimulated by phorbol myristate acetate (PMA), and fixed in 4% formaldehyde. pERK in PBMCs was stained using an antipERK and fluorescein isothiocyanate-conjugated secondary antibody. The cells were resuspended in phosphate-buffered saline and flow cytometry was performed.[12] The plasma concentration of adrenomedullin was measured by immunoradiometric assay at the baseline and on day 28 of the continuous dosing. FDG-PET was performed before treatment, 1, 2, and 3 months after the initiation of treatment, and every 2 months thereafter. The maximum standardized uptake values (SUV_{max}) were recorded. The relationship between trough concentrations of sorafenib on day 28 *versus* SUV_{max} 1 month after the start of continuous dosing was investigated by using an inhibitory Emax model:

Table 3. Incidence of common drug-related adverse events by dose levels

Adverse event	100 mg *n* = 3	200 mg *n* = 15	400 mg *n* = 6	600 mg *n* = 7
Hypertension	0	2 (13%)	1 (17%)	1 (14%)
Fatigue	0	1 (6.7%)	1 (17%)	1 (14%)
Alopecia	0	3 (20%)	3 (50%)	2 (29%)
Dry skin	0	4 (27%)	3 (50%)	0
Hand–foot skin reaction	0	3 (20%)	3 (50%)	6 (86%)
Rash/desquamation	2 (67%)	8 (53%)	6 (100%)	3 (43%)
Pruritus	0	1 (13%)	2 (33%)	2 (29%)
Anorexia	1 (33%)	4 (27%)	1 (17%)	2 (29%)
Diarrhea	0	6 (40%)	3 (50%)	2 (29%)
Lipase	0	4 (27%)	3 (50%)	4 (57%)
Amylase	0	3 (20%)	3 (50%)	2 (29%)

$$E = E_{max} \times (1 - C/[C + EC_{50}])$$

where E is the percentage of SUVmax relative to the baseline, E_{max} is the maximum effect expressed as a percentage of baseline, C is trough concentration, and EC_{50} is the concentration yielding 50% of E_{max}.

Results

Patient characteristics. A total of 31 patients were enrolled in this study: 10 males and 21 females. The median age was 63 years with a range of 32–72 years. The baseline demographics for all patients are shown in Table 1. The commonest cancers were non-small cell lung (10 patients) and colorectal (six patients) cancers. Six of 10 patients with lung cancer had adenocarcinoma. All patients had an ECOG performance status of 0 or 1. Thirty patients had been pretreated with chemotherapy, 29 had had surgery, and 11 radiotherapy. Four patients discontinued treatment during the initial 4-week continuous dosing period (cycle 1) because of disease progression in three and withdrawal of consent in one case. All 31 patients were assessable for safety.

Dose escalations and dose-limiting toxicity. DLTs were not observed in any of the cohort of three patients at 100 mg bid. A total of 15 patients were enrolled at the 200 mg bid dose level, 12 of whom could be evaluated for DLTs (two patients did not complete cycle 1 due to progressive disease and withdrawal of consent in the other). One of these 12 patients presented with grade 3 diarrhea, classified as a DLT. In addition, two patients had grade 3/4 elevations of pancreatic enzymes including grade 4 lipase and grade 3/4 amylase. However, examination of these patients with pancreatic enzyme elevations using ultrasound, magnetic resonance imaging, and computed tomography did not show any evidence of pancreatitis, and the lipase level began to decrease before sorafenib was stopped. After the safety of 200 mg bid had been thus confirmed, the next dose of 400 mg bid was investigated. Six patients in the 400 mg bid cohorts experienced no DLTs, although two had grade 4 lipase elevations which were not associated with pancreatitis. Next, seven patients at 600 mg bid were studied. One patient was taken off the study because of early disease progression. One of the remaining six patients experienced dose-limiting grade 3 fatigue. In addition to this observation, hand–foot skin reactions were observed in five patients at 600 mg bid. Therefore, 400 mg bid sorafenib was established as the MTD and is recommended for future clinical studies.

Safety. Thirty patients experienced drug-related adverse events (Tables 2,3), the most frequent of which were dermatological (77%), gastrointestinal (58%), or elevations of lipase (36%) or amylase (26%). The most common dermatological adverse

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Table 4. Plasma pharmacokinetic parameters of sorafenib

Dose (mg bid)	day 1						day 14			day 28	
	AUC (mg h/L)	AUC_{0-12} (mg h/L)	C_{max} (mg/L)	T_{max} (h)	$T_{1/2}$ (h)	CL/f (L/h)	AUC_{0-12} (mg h/L)	C_{max} (mg/L)	C_{trough} (mg/L)	AUC_{0-12} (mg h/L)	C_{max} (mg/L)
100 ($n = 3$)	9.4	3.3	0.43	4	27.1	10.6	9.4	1.04	0.70	12.3	1.42
	(39)	(42)	(41)	(3–8)†	(39)	(39)	(21)	(30)	(43)	(27)	(35)
200 ($n = 15$)	24.3	5.1	0.74	4	24.4	8.2	20.2‡	2.64‡	1.38§	21.1¶	2.43††
	(100)	(110)	(107)	(3–24)†	(58)	(100)	(37)	(49)	(588)	(49)	(52)
400 ($n = 6$)	35.4	7.0	1.21	8	25.5	11.3	36.7	4.91	3.75	n/a	n/a
	(195)	(173)	(201)	(3–24)†	(40)	(195)	(73)	(76)	(104)		
600 ($n = 7$)	40.5**	9.7	1.41	6	30.4‡‡	14.8‡‡	33.8‡‡	4.42‡‡	4.29‡‡	n/a	n/a
	(67)	(81)	(70)	(4–23)	(34)	(67)	(43)	(55)	(62)		

Data are expressed as geometric mean or median, and percent coefficient of variance is expressed in parentheses.
†range; ‡$n = 10$, §$n = 11$, ¶$n = 8$, ††$n = 9$, ‡‡$n = 6$ (Calculated by using the half of lower limit of quantification for one patient with C_{trough} lower than the lower limit of quantification)
AUC, area under the curve; n/a, not available.

events were rash/desquamation (61%), hand–foot skin reaction (39%), alopecia (26%), dry skin (23%), and pruritus (16%; Table 2). However, these were mild, beginning mostly 2–8 weeks after the start of sorafenib treatment and resolving with the application of local therapies without requiring a change of sorafenib dosing of any patients. No grade 3/4 dermatological toxicities were observed. The incidence of hand–foot skin reaction tended to be dose-dependent (Table 3).

The most common gastrointestinal adverse event was diarrhea (35%). It was mostly mild to moderate and easily managed with oral loperamide. However, grade 3 diarrhea (a DLT) occurred in one patient at the 200 mg bid dose level.

Elevation of lipase or amylase was not observed at the 100 mg bid dose level (Table 3). Of the 15 patients treated with 200 mg bid, four showed elevated lipase (27%) and three elevated amylase (20%). Two of these patients had grade 4 elevated lipase, but no indications of pancreatitis were observed by diagnostic imaging. Three of six patients (50%) in the 400 mg bid group and four of seven (57%) in the 600 mg bid group had elevated levels of pancreatic enzymes, which returned to normal without requiring interruption of sorafenib administration. Serum levels of amylase and lipase began increasing on days 4–7, and then decreased again back to normal levels within 3–10 days with/without stopping administration of sorafenib. No patients had symptoms of pancreatitis. Ultrasound, computed tomography, and magnetic resonance imaging of the pancreas showed no evidence of acute pancreatitis.

Hypertension was observed in four patients, with one occurrence of grade 3 at the 600 mg bid dose level. A causal relationship with the use of the study drug could not be ruled out. These events mostly began 1–7 weeks after the initial sorafenib treatment and returned to normal during continuous treatment thereafter. Treatment-related abnormalities in hepatic parameters, such as ALT and AST elevations, were reported in two patients as serious adverse events, and drug administration had to be discontinued. Fatigue was reported in three patients including one case of dose-limiting grade 3.

Pharmacokinetics. Pharmacokinetics data sets after the initial single dosing were obtained in a total of 31 patients. Thereafter, 25 patients were eligible for pharmacokinetics analysis on day 14 during the continuous dosing; six were excluded because of discontinuation of drug administration. The pharmacokinetic parameters of sorafenib are shown in Table 4. Drug absorption was moderate after the single administration, with time to maximum plasma concentration (T_{max}) 3–24 h (mean, 8 h) after administration. Plasma half-life ($T_{1/2}$) was found to be 24–30 h (mean, 25.5 h). Although considerable interpatient variability was observed, the geometric means of AUC, AUC_{0-12} as well as the maximum and trough concentrations increased dose dependently from 100 mg to 400 mg after administration of a single dose and at steady state (day 14). At 600 mg bid, drug exposure seemed to be increased less than proportionally to the dose escalation. Plasma trough concentrations at 400 mg bid (3.75 mg/L) exceeded the IC_{50} for inhibition of tumor cell proliferation *in vitro* (ranging between 0.057 and 2.5 mg/L).[8]

Major metabolites of sorafenib M-2, M-4, and M-5 were detected in plasma, but the AUC_{0-12} of each metabolite was less than 13% of the sum of all measured compounds (Table 5). Similar to sorafenib, the AUC_{0-12} and C_{max} of these metabolites were increased by dose escalations from 100 to 400 mg bid, but were not further increased at 600 mg bid. Sorafenib and M-2 were not detectable in urine, while the glucuronidated metabolites, M-7 and M-8, were excreted in the urine at up to 4% of the administered dose of sorafenib (Table 6).

Pharmacodynamics. ERK is an essential component of MAPK signaling pathways and a downstream factor of Raf kinase, which is a target molecule of sorafenib.[7,8] Adrenomedullin is a bioactive peptide and known to be expressed/secreted by human tumors.[13,14] In preclinical studies, expression of adrenomedullin decreased in tumors as the plasma concentration of sorafenib increased. Thus, phosphorylation of ERK and plasma adrenomedullin levels may be a candidate biomarker of sorafenib efficacy. Nevertheless, in the present study, large interindividual variations were observed in changes of pERK-positive cells in PBMCs and also in plasma adrenomedullin levels, and no obvious trend was recognizable for these parameters (Table 7).

Twenty-three patients underwent repeated examination by FDG-PET, with the median value of SUV_{max} decreasing significantly from 16.2 (range, 3.0–80.3) at the baseline to 11.2 (3.0–57.8) at the first examination after the start of treatment ($P = 0.0007$ by Wilcoxon signed-rank test). The median percent change from baseline for each patient was –25% (–54% to 25%). SUV_{max} was decreased from baseline in 19 patients, with a 25% or greater decrease being observed in 11 patients. A higher trough concentration of sorafenib on day 28 was associated with larger reduction in SUV_{max} (Fig. 2). This relationship could be described by an E_{max} model with $E_{max} = 130.1$ (SE, 21.0)% and $EC_{50} = 4.8$ (2.4) mg/L.

Antitumor activity. Twenty-nine patients were evaluated for tumor response according to RECIST criteria. Overall duration of treatment was prolonged as the dose was increased. PR was observed in two patients (total, 7%). In a 69-year-old patient with renal cell carcinoma previously treated with interferon-α2b, PR was achieved 1 month after the start of continuous dosing

Table 5. Plasma pharmacokinetic parameters of metabolites

Dose (mg bid)	M-2 (BAY 67-3472)			M-4 (BAY 43-9007)			M-5 (BAY 68-7769)		
	AUC_{0-12} (mg h/L)	Ratio (%)	C_{max} (mg/L)	AUC_{0-12} (mg h/L)	Ratio (%)	C_{max} (mg/L)	AUC_{0-12} (mg h/L)	Ratio (%)	C_{max} (mg/L)
100 ($n = 3$)	0.63	6.07	0.07	0.16	1.54	0.02	0.21†	2.04†	0.02†
	(57)	(74)	(45)	(40)	(25)	(23)	(54)	(78)	(71)
200 ($n = 10$)	2.47	10.01	0.31	0.70	2.83	0.11	0.83‡	3.13‡	0.10‡
	(79)	(55)	(71)	(179)	(124)	(95)	(50)	(63)	(55)
400 ($n = 6$)	5.84	11.7	0.73	1.89	3.81	0.24	1.79	3.60	0.22
	(269)	(63)	(285)	(324)	(81)	(353)	(563)	(144)	(573)
600 ($n = 6$)	5.44	12.2	0.66	1.81	4.09	0.23	1.48	3.34	0.18
	(140)	(58)	(150)	(139)	(61)	(153)	(185)	(84)	(205)

Data are expressed as geometric mean, and percent coefficient of variance is expressed in parentheses.
Ratio of each metabolite to the sum of AUC_{0-12} of sorafenib, M-2, M-4, and M-5.
†$n = 2$; ‡$n = 9$.
AUC, area under the curve.

Table 6. Urinary excretion of sorafenib and metabolites 48 h after single administration of sorafenib

Dose (mg bid)	Sorafaneib (BAY 43-9006) (%)	M-2 (BAY 67-3472) (%)	M-7 (BAY 43-9006G) (%)	M-8 (BAY 67-3472G) (%)
100	ND	ND	4.15 (34)†	0.09 (0)‡
200	ND	ND	1.97 (55)§	0.08 (99)¶
400	ND	ND	1.66 (64)††	0.11 (99)‡‡
600	ND	ND	1.70 (66)††	0.09 (120)††

Percent coefficient of variance is expressed in parentheses.
BAY 43-9006G: BAY 43-9006 glucuronide, BAY 67-3472G: BAY 67-3472 glucuronide.
†$n = 3$, ‡$n = 2$, §$n = 2$, ¶$n = 9$, ††$n = 5$, ‡‡$n = 4$.
ND, not detected.

Table 7. Plasma pharmacodynamics of sorafenib on day 28 of cycle 1

	100 mg ($n = 3$)	200 mg ($n = 12$)	400 mg ($n = 6$)	600 mg ($n = 5$)
pERK+ (%)	44.8 (10.3)	43.6 (15.4)	64.1 (29.6)	57.5 (12.4%)
Adrenomedullin (fmol/mL)	2.18 (0.62)†	1.90 (0.67)	2.97 (1.67)	2.23 (0.61)

Standard deviation is in parentheses.
pERK+ (phosphorylated ERK+) is expressed as percentage of positive cells in peripheral blood mononuclear cells.
†$n = 2$.

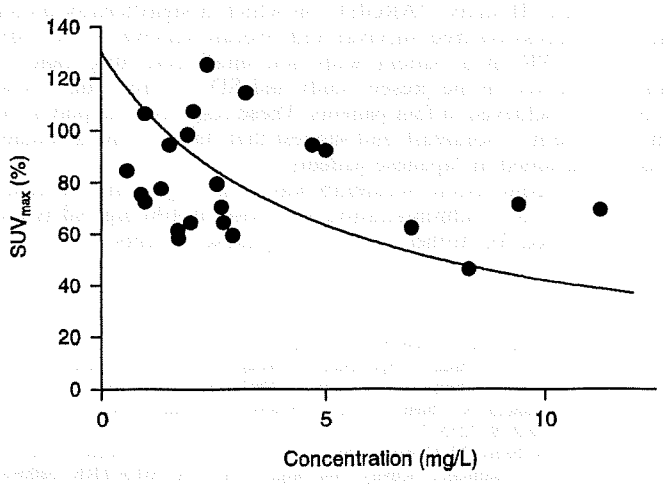

Fig. 2. Relationship between the trough concentration of sorafenib and the maximum standardized uptake value (SUV_{max}) relative to the baseline.

(600 mg bid) and was maintained over 8 months. In another patient with non-small cell lung cancer (NSCLC) who had been treated with cisplatin, vinorelbine, docetaxel, and gefitinib, tumor size gradually decreased and PR was achieved 11 months after the start of continuous dosing (200 mg bid), and was maintained for more than 20 months. Treatment was discontinued when a second cancer (small cell lung cancer) developed, which was surgically resected and treated with cisplatin and etoposide. The original NSCLC did not grow during the treatment course for a period exceeding 30 months. In addition to the PR, a total of 14 patients (48%) experienced SD. Four of 10 patients with non-small cell lung cancer achieved SD for more than 6 months.

Discussion

The results of this study showed a favorable safety profile of sorafenib in Japanese patients with advanced refractory solid tumors. The most common drug-related toxicities including rash/desquamation, hand–foot skin reactions, and diarrhea, and elevations of serum pancreatic enzyme levels were mostly mild to moderate. Dose-limiting toxicities in this study were diarrhea and fatigue.

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Dermatological adverse events were frequently observed. The most common drug-related events were rash/desquamation (61%) and hand–foot skin reactions (39%), which were grade 2 or milder although their incidence was increased with dose escalation from 400 to 600 mg bid (Table 3). Another type of common toxicity was gastrointestinal, such as diarrhea and anorexia. Diarrhea was reported in 11 patients (36%) and one of them experienced a grade 3 dose-limiting event. Previous phase I studies in Europe and the United States in patients with advanced refractory solid tumors (100–800 mg bid) showed similar drug-related adverse events.[15–19] The most frequently reported adverse events in four studies were fatigue (40%), anorexia (35%), diarrhea (34%), rash/desquamation (27%), and hand–foot skin reactions (25%). Similarly, the incidence rates of these drug-related adverse events were higher in the 600 mg group. Diarrhea and fatigue were also dose-limiting toxicities in those studies, and the most common drug-related events were dermatological and gastrointestinal toxicities, which were comparable between Japanese and non-Japanese patients.[15–19] Similar to the previous phase I studies, the results of this study suggests that it is appropriate to recommend 400 mg bid for phase II studies in Japan.

Elevated lipase (36%) and amylase (26%) levels were also common drug-related adverse events, and seven patients (23%) experienced grade 3 or worse. The incidences seemed to be dose-dependent, suggesting that it was related to sorafenib. In a preclinical study, histological changes in the pancreas were observed. Such elevations have been rarely reported in previous clinical studies of sorafenib performed in other countries, where pancreatic enzyme levels were not routinely measured. Lack of symptoms and the transient nature of this toxicity could have led to underestimation in previous studies. The elevation of lipase was also reported in patients treated with sunitinib, a receptor tyrosine kinase inhibitor,[20] which inhibits VEGFR-2, PDGFR, Flt-3, and c-KIT.[21,22] The mechanism of the elevation of pancreatic enzymes may be related to kinase inhibition or to some chemical property, rather than to inhibition of angiogenesis, because patients treated with bevacizumab, an anti-VEGF antibody, did not experience this.[23,24] Importantly, elevations of pancreatic enzyme levels did not cause any clinically relevant events. They were transient, and did not interrupt the sorafenib administration schedule in most patients in the present study. However, as pancreatitis was reported in other clinical trials of sorafenib,[25] physicians treating patients with this drug need to recognize the possibility of occurrence of pancreatitis, although the diagnosis of pancreatitis should not be made solely on the basis of elevation of pancreatic enzymes.

The results of pharmacokinetic analysis suggested that the AUCs of sorafenib and metabolites were related to dose within the range of 100–400 mg bid, but with no further increase at 600 mg. Although the N-oxide of sorafenib (M-2) is the main drug metabolite in plasma, sorafenib exists in plasma mostly in an unchanged form. The ratio of the metabolite to the sum of the unchanged drug and three metabolites was 6–12%, which was lower than the 17% measured in healthy volunteers who received [^{14}C]-sorafenib.[11] The difference might be related to variation in subjects, methodology, and the dose.

Preclinical data suggested that sorafenib is metabolized by CYP3A4. However, coadministration of ketoconazole, a CYP3A4 inhibitor, did not change the concentration of sorafenib in healthy volunteers. In this case, the formation of the main metabolite decreased, suggesting other metabolic pathways, such as glucuronidation.[11] This is the first report that has investigated urinary excretion of sorafenib and metabolites in cancer patients. It was found that glucuronidated sorafenib and other glucuronidated metabolites but not sorafenib itself were in fact excreted in the urine. The amount of metabolites excreted in urine was 2–4%. Following oral administration of [^{14}C]-sorafenib to healthy volunteers, 19% of the dose was excreted as glucuronides in urine, and 77% in feces (50% as unchanged drug).[11] A gain, variation in subjects, methodology, and the dose might explain the difference in the amount of drug excreted in urine. Consistent with the results of previous phase I studies in non-Japanese patients, considerable interpatient variability in relation to the pharmacokinetics of sorafenib was observed in Japanese patients as well.[15] Although drug exposures in Japanese patients were slightly lower than in non-Japanese patients,[15] available data suggest that no dosage adjustment due to ethnicity will be necessary.

We assessed pharmacodynamics in patients treated with sorafenib. ERK is a downstream kinase of Raf kinase, and when sorafenib inhibits Raf kinase, the phosphorylation levels of ERK may also be decreased.[8] Previous clinical studies indicated a significant reduction of pERK levels with increasing sorafenib dose.[18] In the present study, pERK-positive cells within PBMCs were not found to change at any of the dose tested. In addition, adrenomedullin was suggested to be a biomarker of sorafenib in preclinical studies, but no significant changes were observed in our clinical study. In contrast, FDG-PET analysis, performed one month after the start of continuous dosing, showed that treatment with sorafenib decreased disease activity in 83% of patients. Furthermore, reduction in FDG uptake was associated with drug exposure. These observations imply that FDG-PET may be used as a surrogate endpoint. Validity of FDG-PET in evaluating the activity of molecular targeted drugs needs to be further investigated.

Preliminary efficacy data in this study indicated one confirmed PR in a patient with renal cell carcinoma. Angiogenesis is suggested as an essential factor in the progression and metastasis of the disease.[26] The anti-VEGF antibody bevacizumab inhibits VEGF signalings and has demonstrated antitumor activity against renal cell carcinoma. Sorafenib targets VEGFR-2 and PDGFR and inhibits angiogenesis. The efficacy of sorafenib for renal cell carcinoma has been demonstrated in a clinical phase III study (TARGET), in which it significantly prolonged progression-free survival and overall survival.[9] In addition, one PR in a patient with non-small cell lung cancer was observed in the present study and SD for more than 24 weeks was achieved in four patients. These responses support a clinical benefit of sorafenib and suggest that further clinical studies are warranted in Japanese patients.

In conclusion, sorafenib was generally well tolerated, and continuous administration at a dose of 400 mg bid is recommended for further studies in Japanese patients.

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