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ORIGINAL ARTICLE

Phase I, pharmacokinetic, and biological studies of TSU-68, a novel multiple receptor tyrosine kinase inhibitor, administered after meals with solid tumors

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Abstract
Purpose TSU-68 is a low molecular weight inhibitor of the tyrosine kinases for vascular endothelial growth factor receptor 2, platelet-derived growth factor receptor β, and fibroblast growth factors receptor 1. In this study, we assessed the recommended dose with TSU-68 administration of twice-daily (b.i.d.) or thrice-daily (t.i.d.) after meals for 4 weeks in Japanese patients with solid tumors based on the safety and tolerability and investigated the relationship between angiogenesis biomarker and clinical outcomes.
Methods The study design was a dose-escalation method with alternating enrollment of b.i.d. administration and t.i.d. administration after meal by traditional three-patient cohort.
Results We enrolled 24 patients at doses of 200, 400, and 500 mg/m^2 b.i.d. or 200 and 400 mg/m^2 t.i.d. No dose-limiting toxicity (DLT) occurred in the 200 mg/m^2 b.i.d. or t.i.d., and 3 patients experienced DLTs at 400 mg/m^2 b.i.d. or 400 mg/m^2 t.i.d. As main toxicity, blood albumin decreased, malaise, diarrhea, alkaline phosphatase increased, anorexia, abdominal pain, nausea, and vomiting were observed as almost all grade 1–2. There were no apparent differences in pharmacokinetic parameters between days 2 and 28 after the repeated b.i.d. and t.i.d. doses. Although tumor shrinkage was not observed, the disease control rate was 41.7%. As an angiogenesis-related factor of stratified analysis, plasma vascular endothelial growth factor and plasminogen activator inhibitor-1 were detected as a significant increase with progressive disease patients.
Conclusions A recommended dosage of TSU-68 for this administration schedules was estimated to be 400 mg/m^2 or less b.i.d.

Keywords Receptor tyrosine kinase inhibitor · Solid tumors · Phase I · Pharmacokinetic

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Introduction

Angiogenesis is essential for the proliferation of malignant tumors and development of its metastasis [1]. When a tumor grows to be 2–3 mm or more in the course of proliferation, it may produce angiogenesis-stimulating growth factors by acting on itself and its surrounding normal cells to supply oxygen and nutrition. Such growth factors may induce digestion, migration/proliferation, and formation of lumens of the basement membrane of endothelial cells, leading to formation of a new vascular nest. This may enlarge the lesion, resulting in infiltration

and hematogenous metastasis. There are many known growth factors for angiogenesis, including vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), and fibroblast growth factor (FGF). Among them, VEGF is regarded as more important since it is reported that the production of VEGF may be increased in many solid tumors and this ability to produce may be correlated with the number of blood vessels and prognosis in breast caner [2], gastric cancer [3], colon cancer [4], lung cancer [5], and others. Angiogenesis by VEGF starts when a VEGF is bound with a VEGF receptor that is a specific receptor appearing in endothelial cells. Subsequently, a VEGF receptor will transmit signals for proliferation and such like following autophosphorylation by tyrosine kinase of an intracellular domain. VEGF receptors comprise Flk-1/KDR, Flt-1, and Flt-4 [6]. In particular, Flk-1/KDR is considered indispensable for the proliferation of endothelial cells as the most important receptor, only appearing in endothelial cells [7]. Neutralization antibodies of VEGF and inhibitors of Flk-1/KDR inhibited proliferation of endothelial cells in vitro, and also suppressed angiogenesis and tumor proliferation in vivo [8, 9]. It is also shown that cellular proliferation was suppressed and tumors had fewer vessels when cell strains derived from ovarian cancer, lung cancer, and glioma were subcutaneously transplanted into SCID mice with a manifestation of Flk-1, the variant lacking tyrosine kinase domain [10, 11].

It has been realized that the susceptibility of established tumor blood vessels to an interference with VEGF/VEGFR2 signaling may be restricted to a fraction of immature vessels that lack co-localization with pericytes. The contact between endothelial cells and periendothelial support cells, such as pericytes or smooth muscle cells, stabilize new blood vessels, promotes endothelial survival, and inhibits endothelial cell proliferation. This is supported by the observation that interference with PDGF-BB/PDGFRb signaling resulted in disruption of already established endothelial/pericytes associations and vessel destabilization [12, 13].

TSU-68 (chemical name: (Z)-5-[(1, 2-dihydro-2-oxo-3H-indol-3-ylidene) methyl]-2,4-dimethyl-1H-pyrrole-3-propanoic acid) is a new, oral angiogenesis inhibitor. It has a low molecular weight that cuts the supply of oxygen and nutrition by inhibiting tyrosine phosphorylation of Flk-1/KDR, VEGF receptor and suppressing angiogenesis in tumor tissues to suppress tumor proliferation and metastasis [14]. In addition, it was confirmed in pre-clinical studies that the substance inhibited tyrosine phosphorylation of PDGF and FGF receptors that are associated with the transmission of intracellular signals as well as VEGF receptors [15, 16], and also inhibited these related angiogenesis in mice [17].

This study was conducted on Japanese patients with advanced solid tumors to evaluate adverse events and to estimate the recommended dose for twice-daily (b.i.d.) or thrice-daily (t.i.d.) administration after meals for a 4-week treatment of TSU-68. This study was designed to comply with the ethical principals of Good Clinical Practice in accordance with the Declaration of Helsinki.

Patient and methods

Patient selection

Patients with solid tumors whose malignancies were confirmed histopathologically, and patients with malignancies on which the standard therapy had no effect or for which no generally approved standard therapy exists, from a single institution in Japan. They were required to be 20–75 years old, can take the drug orally, have performance statues of 0–2, and expected to survive for a sufficient period of $\geq$60 days. Patients with physiologically adequate compensatory functions and with parenchymal organs, such as heart, pulmonary, renal, and bone marrow, in particular, functioning sufficiently, were eligible for this study. The following laboratory values, obtained within 15 days before the start of the study, must be satisfied (Leukocyte count: 4,000–12,000/mm^3 or neutrophil count: $\geq$2,000/mm^3, platelet count: $\geq 10 \times 10^4$/mm^3, hemoglobin level: $\geq$8.0 g/dl or more, total bilirubin: $\leq$1.5 mg/dl, glutamic-oxaloacetic transaminase/glutamic-pyruvic transaminase: $\leq$100 U/l, serum creatinine: $\leq$1.5 mg/dl, creatinine clearance: $\leq$50 ml/min, PaO_2:$\geq$65 mmHg, no clinical evidence of abnormality on electrocardiogram). These patients needed to have confirmed measurable lesions.

Patients were excluded for the potential influence of previous treatments, such as major invasive surgery, blood transfusion, or administration of G-CSF. Patients with active infections, serious complications, a history of serious thromboembolism, brain metastasis showing clinical symptoms, and colon diseases accompanied by active inflammation were excluded from this study.

It was confirmed that the investigator explained the details of the study to the patients in accordance with the information form before enrollment, and then allowed sufficient time before obtaining written consent. Prior to the conduct of this study, it was reviewed by the institutional review board at the National Cancer Center.

Drug administration

TSU-68 was provided by Taiho Pharmaceutical Co., Ltd (Tokyo, Japan). Twice-daily administration was given within 1 h after meals at about 12 h interval by a dose

corresponding to each specified dosage level per body surface area, and thrice-daily administration was given at about 6- or 12-h interval.

TSU-68 was taken for 28 days continuously, and for patients evaluated as being better in evaluation of antitumor effect after completion of 28 days, the administration was to be continued within the range of this study period, unless it became difficult to continue the treatment due to occurrence of any adverse event.

Dose escalation

The dose escalation was a three-patient cohort at each dose level and alternating of b.i.d. and t.i.d. The starting dose and number of TSU-68 was 200 mg/m^2 b.i.d.

Dose-limiting toxicity (DLT) was defined as drug-related adverse events (adverse drug reactions) of grade 3 or more severe non-hematological toxicity or grade 4 or more severe hematological toxicity. The doses were increased by 100% for patients showing no adverse drug reaction of grade 2, by 40% for cases showing no DLT and adverse drug reaction of grade 3, or by 33% for cases showing DLT.

Each dosage level involved 3 patients, and 3 additional patients were to be administered if one or more of the initial three patients showed DLT. The drug was to be administered to at least 6 patients at the recommended dosage level. The maximum tolerated dose (MTD) was the appearance of DLT in 2/3 patients or 3/6 patients, and provided that those DLT was counted by the same toxicity category. The recommended dosage was determined to be one level lower than the dosage judged as the MTD. If the MTD was not found, the recommended dosage was also determined in consideration of the results of the pharmacokinetic investigation.

Patient evaluation

Patient condition was assessed by hematology/chemistry laboratory data, urinalysis, vital signs, performance status, and clinical findings at least weekly. Symptoms were evaluated in accordance with the Common Toxicity Criteria version 2.0 (NCI-CTC) [18].

Antitumor effects and adverse reactions were evaluated in accordance with the criteria of the Japan Society for Cancer Therapy [19], which is based on criteria established by the WHO. The criteria for the evaluation of antitumor effects were as follows: complete response (CR), eradication of all cancers and maintenance of the condition for 4 weeks or more; partial response (PR), 50% or more reduction in size of lesions and maintenance of the condition for 4 weeks or more; no change (NC), less than 50% reduction in size of lesions or enlargement of lesions within 25% and maintenance of the condition for 4 weeks or more; progressive disease (PD), 25% or more enlargement of lesions or appearance of new lesions.

Pharmacokinetics studies

For twice-daily administration, sequential blood collection was performed after the 1st, 3rd, and 55th doses. Blood samples were collected within 30 min before dosing and at the following times after dosing: 1, 1.5, 2, 3, 4, 6, 8, and 12 h (the 1st, 3rd, and 55th doses), and l, 1.5, 2, 3, 4, and 12 h (the 56th dose).

For three times daily administration, sequential blood collection was performed after the 1st dose and the 2nd, 82nd, and 84th doses (respective about 6, 12 and 6 h after prior dose). Blood samples were collected within 30 and 10 min before dosing for the 1st and 3rd doses, and at the following times after dosing: 1, 1.5, 2, 3, 4, and 5.5 h (the 1st and 82nd doses); 1, 1.5, 2, 3, 4, and 6 h (the 2nd dose); and 1, 1.5, 2, 3, 4 and 14 h (the 84th dose).

Urine samples were collected at the following intervals for measurement of TSU-68: For twice-daily administration, prior to the 1st dose, 0–12 h after the 1st, 2nd, 3rd, 15th, 55th doses, and 0–12 and 12–24 h after the 56th dose. For three times daily administration, prior to the 1st dose, 0–6 h after the 1st, 2nd, 22nd, 82nd, and 83rd doses, 0–12 h after the 3rd dose, and 0–6, 6–14 and 14–24 h after the 84th dose.

TSU-68 concentration was determined using a validated high-performance liquid chromatography method with UV detection, with a lower limit of quantification of 0.1 μg/ml.

In pharmacokinetic analyses, non-compartmental pharmacokinetic parameters including area under the plasma concentration–time curves (AUC) from time 0 to the last measurable time (AUC_{0-t}), maximum concentration (C_{max}), time to maximum concentration (T_{max}), and elimination half-life ($T_{1/2}$) were calculated using PhAST (Ver.2.3, MDS Pharma Services, Montreal, Canada).

Biological studies

As for plasma and urine collection before treatment and after day 8 and day 28, plasma VEGF, urinary VEGF, endothelial adhesion molecule-1 (ELAM-1), tissue plasminogen activator (t-PA), plasminogen activator inhibitor-1 (PAI-1), and vascular cell adhesion molecule-1 (VCAM-1) were measured at SRL, Inc. (Tokyo, Japan). Enzyme-linked immunosorbent assay (ELISA) kits for human VEGF, human ELAM-1, and human VCAM-1 were obtained from QuantikineTM from R&D Systems Inc. (Minneapolis, MN). ELISA kit for soluble t-PA was obtained from Calbiochem (La Jolla, CA). Latex photometric immunoassay system for soluble PAI-1 was obtained from Mitsubishi

Chemical Medicine Corporation (Tokyo, Japan). The analysis was performed using the SAS®.

Results

Patient characteristics

Twenty-four patients were enrolled in this study between June 2001 and March 2002. All patients were evaluable for safety, pharmacokinetics, and biological studies. The patient characteristics are summarized in Table 1. All patients had a good performance status, and their median age was 55 years (range 31–72 years). The site of the primary tumor was colorectal cancer in 9 patients, non-small-cell lung cancer in 7 patients, and others in 8 patients. Twenty-two patients had received prior systemic chemotherapy with standard regimen, and 2 patients had no standard therapy.

Maximum tolerated dose

Patients were enrolled sequentially on the twice-daily/thrice-daily administration cohorts and in parallel within each dosing cohort. No DLT occurred in the 200 mg/m^2 b.i.d dose level (3 patients). Three plus three patients were enrolled on the 400 mg/m^2 b.i.d. dose level, with 2 patients experiencing DLTs: grade 3 dyspnea, hypoxemia, pleural effusion and anorexia, and unacceptable grade 2 anorexia. The grade 2 anorexia at 400 mg/m^2 was an excruciating event with weight loss and blood albumin decrease, and this patient refused drug administration after 6 days. Two patients of DLT in 400 mg/m^2 were different toxicity categories. One patient was Pulmonary (grade 3 of dyspnea, hypoxemia, pleural effusion, and anorexia, anorexia were the accompanying events of dyspnea), and the other patient was Gastrointestinal (anorexia of grade 2). Therefore, it did not count 2/3 patients, and enrolled patients with total six at 400 mg/m^2. However, the 500 mg/m^2 b.i.d. dose level was

Table 1 Patient characteristics

Pt	Age	Sex	PS	BSA (m^2)	Diagnosis	Frequency	Dosage		AUC Day 28	Toxicity ≥ G3-4	Best response (TTP, days)
							(mg/m^2)	(mg/day)[a]			
1	54	F	1	1.75	Cervical ca.	b.i.d.	200	800	32.8	–	NC (162)
2	54	M	1	1.66	NSCLC	b.i.d.	200	800	33.4	–	NC (64)
3	31	F	1	1.43	Parotid ca.	b.i.d.	200	400	30.4	–	PD
4	72	F	0	1.37	SCLC	b.i.d.	400	1,200	–	G3 (DLT)	NC (29+)
5	55	F	1	1.34	NSCLC	b.i.d.	400	1,200	–	– (DLT)	NE
6	50	F	1	1.43	Unknown	b.i.d.	400	1,200	54.8	–	PD
7	53	M	1	1.78	NSCLC	b.i.d.	400	1,600	54.0	–	PD
8	47	M	1	1.71	Cholecystis ca.	b.i.d.	400	1,200	21.6	–	NC (61)
9	47	M	1	1.68	NSCLC	b.i.d.	400	1,200	17.1	–	NC (29+)
10	60	F	1	1.48	Colon ca.	b.i.d.	500	1,600	61.2	–	PD
11	63	F	1	1.67	Colon ca.	b.i.d.	500	1,600	44.6	–	PD
12	66	F	1	1.37	NSCLC	b.i.d.	500	1,200	23.8	–	PD
13	46	M	1	1.59	Colon ca.	t.i.d.	200	1200	25.3	–	PD
14	51	F	1	1.31	Soft tissue sa.	t.i.d.	200	600	19.0	–	NC (254+)
15	57	M	1	1.60	Rectal ca.	t.i.d.	200	1,200	51.0	–	PD
16	50	F	1	1.77	Colon ca.	t.i.d.	200	1,200	24.0	–	PD
17	62	M	1	1.70	NSCLC	t.i.d.	200	1,200	16.6	–	NC (65)
18	60	M	1	1.70	Colon ca.	t.i.d.	200	1,200	12.5	–	PD
19	56	M	1	2.26	NSCLC	t.i.d.	400	2,400	9.9	G4 (DLT)	NC (28+)
20	54	F	1	1.51	Colon ca.	t.i.d.	400	1,800	46.8	–	NC (217+)
21	64	M	1	1.91	Colon ca.	t.i.d.	400	2,400	27.8	–	PD
22	51	M	1	1.68	Gastric ca.	t.i.d.	400	1,800	21.3	–	PD
23	59	F	1	1.37	Colon ca.	t.i.d.	400	1,800	30.7	–	NC (28+)
24	55	M	0	1.68	Esophagus ca.	t.i.d.	400	1,800	53.4	–	PD

PS performance status, *BSA* body surface area, *AUC* area under the curve, *G* grade, *NC* no change, *PD* progressive disease, *ca* cancer, *sa* sarcoma, *NSCLC* non-small-cell lung cancer, *DLT* dose-limiting toxicity, *TTP* time to progression, *b.i.d.* TSU-68 administration of twice-daily, *t.i.d.* TSU-68 administration of thrice-daily

[a] One tablet: 200 mg

not found to DLT, and MTD was not reached, because dose escalation was stopped based on pharmacokinetic results. On the other hand, no DLT occurred in the 200 mg/m^2 t.i.d. dose level, and one patient experienced a DLT of grade 4 pericardial effusion at 400 mg/m^2 t.i.d. dose level. MTD was not reached either because dose escalation was not based on the result of pharmacokinetics, and three patients were enrolled in each dose level.

Toxicity

All 24 patients were evaluated for safety analysis. Major drug-related adverse events for 4-week administration are shown in Table 2. As protocol-defined DLT, there were two patients who had grade 4 pericardial effusion by t.i.d., and grade 3 dyspnea, hypoxemia, pleural effusion, and anorexia by b.i.d. These adverse events were not the defined DLT with revealed characteristics of the TSU-68 safety profile. The main toxicities were almost all grade 1–2, and the toxicities occurring in at least over 30% included urine/stool discoloration, blood albumin decrease, fatigue, diarrhea, blood alkaline phosphatase increase, anorexia, abdominal pain, nausea, and vomiting.

Pharmacokinetics

In the b.i.d. regimen after meal, pharmacokinetic analyses were performed in 12 subjects, at the doses of 200 mg/m^2 ($n = 3$), 400 mg/m^2 ($n = 6$), and 500 mg/m^2 ($n = 3$). In the t.i.d. regimen after meal, pharmacokinetic analyses were performed in 12 subjects, at the doses of 200 mg/m^2 ($n = 6$) and 400 mg/m^2 ($n = 6$). The mean concentration–time profiles in each dose level are shown in the Fig. 1. Pharmacokinetic results are presented in Table 3.

In the b.i.d. and t.i.d. regimens after meal, after the 1st dose, the plasma concentration of TSU-68 increased to reach C_{max} at approximately 3 h, and thereafter disappeared with $T_{1/2}$ of approximately 2–3 h. In the b.i.d. regimen, at any dose levels, C_{max} and AUC after the repeated administration of TSU-68 on days 2 and 28 were approximately twofold lower than those after the 1st administration on day 1. The t.i.d. regimen also shows a similar trend. There were no apparent differences in these parameters between days 2 and 28. In addition, no obvious dose-dependent increases were observed with these parameters after the repeated b.i.d. and t.i.d. doses.

Urinary excretion of TSU-68 was below 1% of dose in all dose levels.

Efficacy

Clinical response was estimated by 23 patients with one patient receiving medication of 1 cycle for less than 50%, and the best response of each case is indicated in Table 1. Although a PR patient was not found, the NC patients were observed at 43.5% (10/23 example) in these studies. Notably, two patients with soft tissue sarcoma (Pt.14) and colon cancer (Pt.20) were treated with TSU-68 for 6 and 9 months. In addition, the median time to progression was 28 days (range 27–254+ days), and the median survival period was 218 days (range 79–465+ days).

Biomarkers

As an angiogenesis-related factor, the plasma VEGF, urinary VEGF, ELAM-1, t-PA, PAI-1, and VCAM-1 were investigated in 23 patients at baseline, 23 patients at day 8, and 22 patients at day 28. Median (range) of those factors were as follows: plasma VEGF to 66 pg/ml (<31–183 pg/ml) at baseline, 55 pg/ml (<31–278 pg/ml) at day 8, and 63 pg/ml (<31–270 pg/ml) at day 28; urinary VEGF to 108 pg/ml (<31–491 pg/ml) at baseline, 144 pg/ml (<31–1210 pg/ml) at day 8, and 162.5 pg/ml (68–730 pg/ml) at day 28; ELAM-1 to 51 ng/ml (14–134 ng/ml) at baseline, 50 ng/ml (13–136 ng/ml) at day 8, and 45.5 ng/ml (17–114 ng/ml) at day 28; t-PA to 7.2 mg/ml (2.6–15.4 ng/ml) at baseline, 7.2 ng/ml (3.3–14.3 ng/ml) at day 8, and 6.8 ng/ml (2.9–13.5 ng/ml) at day 28; PAI-1 to 23 ng/ml (10–73 ng/ml) at baseline, 28 ng/ml (11–92 ng/ml) at day 8, and 31 ng/ml (11–120 ng/ml) at day 28; VCAM-1 to 501 ng/ml (373–1,080 ng/ml) at baseline, 562 ng/ml (303–1,140 ng/ml) at day 8, and 545.5 ng/ml (355–988 ng/ml) day 28.

As a result of stratified analysis on efficacy (separated between NC of 10 pts. and PD of 13 pts.) before and after treatment, plasma VEGF and PAI-1 were detected as a significant change by Wilcoxon signed rank-sum test. Figure 2 shows the change in these factors for each patient. Both factors with PD patients were significantly increasing at day 28 compared to baseline. Three progressors in VEGF were the same patients of 3 progressors in PAI-1, and these 3 progressors were Pt.11, Pt.13, and Pt.18 in Table 1. Regarding the other factors, no significant change was found in NC patients or PD patients. Also, the angiogenesis-related factors were compared with NC patients and PD patients on baseline, but a factor in a significant correlation with clinical efficacy could not be detected.

Discussion

This study investigated the difference of toxicity and pharmacokinetic data between twice-daily and thrice-daily administering after meal, based on a pre-clinical study, which plasma concentrations of TSU-68 orally

Table 2 Number of patients with drug-related adverse events

Adverse events	b.i.d.												t.i.d.								Total no. (%) $n = 24$
	200 mg/m^2 × 2 $n = 3$				400 mg/m^2 × 2 $n = 6$				500 mg/m^2 × 2 $n = 3$				200 mg/m^2 × 3 $n = 6$				400 mg/m^2 × 3 $n = 6$				
	G1	G2	G3	G4	G1	G2	G3	G4	G1	G2	G3	G4	G1	G2	G3	G4	G1	G2	G3	G4	
Discoloration urine/stool	3				6				3				6				6				24 (100)
Blood albumin decreased	1	1			2	2			1	2			5	1			3	2			20 (83.3)
Malaise	1	1			4				3				6				3				18 (75.0)
Diarrhea	1	1			5				1				4	1			3	1			17 (70.8)
Alkaline phosphatase increased	1				6				1				2				2	1			13 (54.2)
Anorexia	2				2	1	1		2				2				1	1			12 (50.0)
Abdominal pain		2			1	2							1	2			1	2			11 (45.8)
Nauseous		1			2	1			3				1				1				9 (37.5)
Vomiting		1			2				2				3								8 (33.3)
Headache					1	2			1	1							2				7 (29.2)
Serum total protein decreased	1					1			1	1			1				1				7 (29.2)
γ-glutamyltransferase increased					1	2							1				2				6 (25.0)
Face edema									1				2				3				6 (25.0)
Back pain					1	1								1				2			5 (20.8)
Abdominal pain upper					1				1	1				2							5 (20.8)
Pleural effusion					1		1		1				1				1				5 (20.8)
Dyspnea							1														1 (4.2)
Hypoxia							1														1 (4.2)
Pericardial effusion																				1	1 (4.2)
No. maximum grade	0	3	0	0	0	5	1	0	0	3	0	0	1	5	0	0	0	5	0	1	

b.i.d. TSU-68 administration of twice-daily, *t.i.d.* TSU-68 administration of thrice-daily, *G* grade

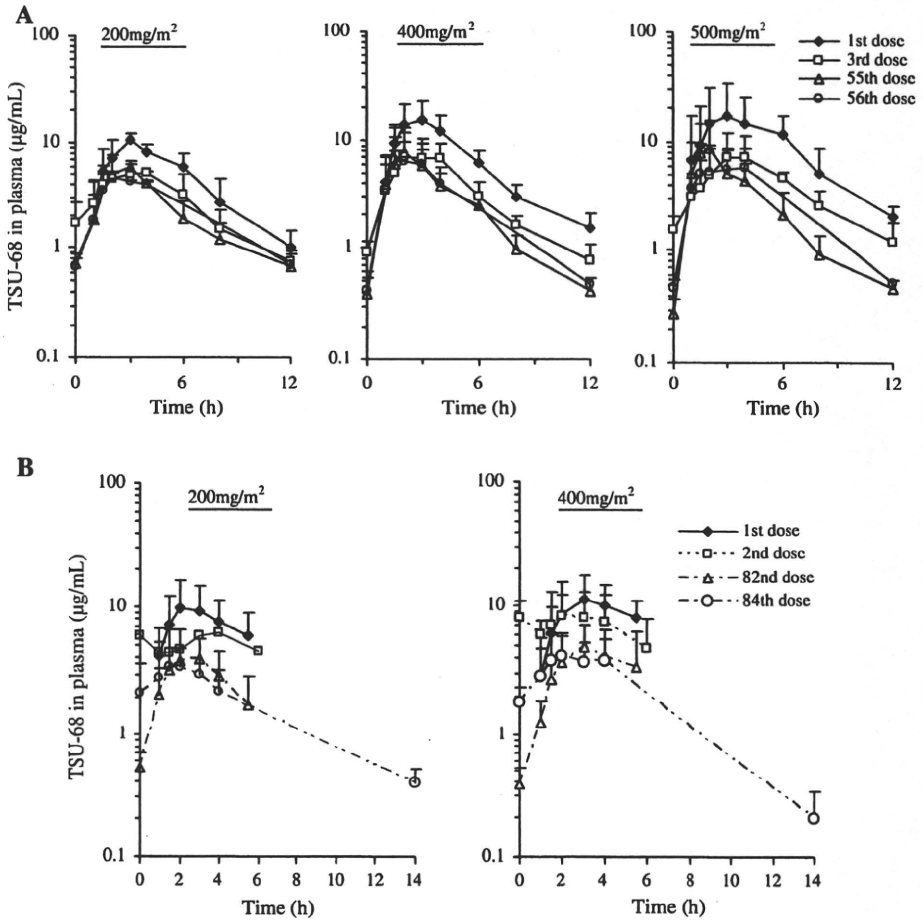

Fig. 1 Plasma concentration-versus-time profiles of TSU-68. **a** Twice-daily administration after meal, **b** thrice-daily administration after meal

administered to fed dogs were higher than that administered to fasted dogs.

In this study, in the time before the advent of molecular-targeted agent, the completed standards systemic chemotherapy population was appropriate for patient evaluation of TSU-68 toxicity and feasibility. The subjects of the protocol-defined DLT were two patients by twice-daily administration and one patient by thrice-daily administration. The plasma concentrations of TSU-68 after day 28 were hardly increased with doses from 200 mg/m^2 b.i.d. to 400 mg/m^2 t.i.d. For this reason, the dose escalation should stop at 1,200 mg/m^2 per total daily dose, and the determination of an unreached MTD was reasonable.

As the main toxicities of TSU-68 in this study, malaise, diarrhea, anorexia, abdominal pain, nausea, and vomiting were observed as subjective symptoms, except the urine/stool discoloration from drug colors. Almost all of the toxicities were grades 1–2, and there were no remarkable differences in the adverse events and grades of side effects by the medication method or the dose level. Moreover, many events of blood albumin decreased and alkaline phosphatase increased as laboratory test values were observed, but there was little evidence of myelosuppression. Consequently, these toxicities were considered acceptable for an oral molecule targeting agent.

The toxicity of TSU-68 did not demonstrate gastrointestinal perforation of characteristic of monoclonal VEGF antibody [20], or a high incidence of a hypertension or skin rash in VEGFR tyrosine kinase inhibitors [21, 22]. On the other hand, as a toxicity characteristic of TSU-68, fluid retention such as face edema, pleural effusion, and pericardia effusions were seen with a relatively high frequency. As similar characteristics, tyrosine kinases inhibitor of a high incidence of fluid retention was Imatinib, targeted for Bsc-Abl, KIT and PDGFR, and was reported at 54% [23].

As a biomarker, although the factor relevant to angiogenesis was measured before and after TSU-68 administration, no factor changed significantly. When this biomarker was stratified by NC patients and PD patients, VEGF and PAI-1 increased significantly after TSU-68 administration compared with before administration in PD patients. These stratification factors suggest the possibility that a significant increase in both VEGF and PAI-1 become response markers of early tumor progression for TSU-68. It

Table 3 Summary of TSU-68 pharmacokinetic data

Dose	mg/m^2 (n)	T_{max} (h)	C_{max} (mg/ml)	AUC_{0-t} (h)	$T_{1/2}$ (h)
b.i.d.					
1st	200 (3)	3.000 ± 1.000	11.213 ± 1.1470	55.19 ± 5.356	2.365 ± 0.8600
	400 (6)	2.667 ± 0.8165	17.088 ± 6.5872	76.44 ± 23.07	2.935 ± 0.6925
	500 (3)	4.000 ± 1.732	22.538 ± 10.019	102.8 ± 45.57	2.351 ± 0.5992
3rd	200 (3)	3.500 ± 2.291	6.0000 ± 0.67065	33.28 ± 4.329	2.707 ± 0.4712
	400 (6)	2.667 ± 0.8165	8.2905 ± 2.7224	40.38 ± 10.63	3.450 ± 1.366
	500 (3)	4.333 ± 1.528	8.7873 ± 2.9245	45.74 ± 14.59	3.334 ± 1.161
55th	200 (3)	2.000 ± 0.8660	6.4597 ± 1.9125	28.71 ± 1.601	3.453 ± 0.9825
	400 (6)	2.250 ± 0.5000[a]	8.5832 ± 4.5463[a]	31.97 ± 11.32[a]	2.620 ± 0.4410[b]
	500 (3)	3.333 ± 2.309	9.3587 ± 5.7174	34.09 ± 12.44	2.958 ± 0.7176
56th	200 (3)	2.667 ± 1.155	4.7870 ± 0.52566	32.20 ± 1.543	3.469[c]
	400 (6)	2.375 ± 0.7500[a]	8.4130 ± 5.8740[a]	36.86 ± 20.31[a]	2.595 ± 0.5267
	500 (3)	3.167 ± 1.443	7.5103 ± 3.4614	43.19 ± 18.72	1.820[d]
t.i.d.					
1st	200 (6)	3.083 ± 1.429	10.997 ± 5.5284	37.04 ± 18.24	2.035 ± 0.4607[b]
	400 (6)	3.417 ± 1.201	12.450 ± 5.7597	41.55 ± 21.60	2.610[d]
2nd	200 (6)	3.667 ± 1.366	7.0053 ± 2.4124	31.52 ± 12.52	2.986[c]
	400 (6)	3.000 ± 1.789	9.3387 ± 4.5090	42.01 ± 18.90	2.406 ± 1.144[b]
82nd	200 (6)	2.667 ± 0.983	4.6755 ± 1.4910	15.16 ± 4.886	1.459[c]
	400 (6)	3.833 ± 1.438	6.0472 ± 2.4067	17.31 ± 6.867	1.990[d]
84th	200 (6)	2.000 ± 1.049	3.8457 ± 2.9842	24.75 ± 13.69	4.773 ± 2.221[e]
	400 (6)	2.917 ± 1.201	4.7810 ± 1.9514	31.65 ± 16.13	2.226[c]

(Mean ± SD, $N = 6$: 200 mg/m^2 of t.i.d. and 400 mg/m^2, $N = 3$: 200, 500 mg/m^2 of b.i.d.)

[a] $N = 4$, [b] $N = 3$, [c] $N = 2$, [d] $N = 1$, [e] $N = 5$

is known that VEGF participates in the blood vessel rebirth of tumor multiplication [5], and PAI-1 was increased by the effect of PDGF in a vascular smooth muscle cell [24, 25]. Since the changes of PAI-1 levels show the difference between NC patients and PD patients, there is some potential for inhibitory activity in PDGFR tyrosine kinases by TSU-68. However, as the values of the biomarkers prior to the administration were not related by NC patients and PD patients stratification, a marker could not be used to predict the clinical effect for TSU-68.

The inhibition of PDGFR tyrosine kinase by TSU-68 enforced tumor vessel regression by interfering with pericyte-mediated endothelial cell survival [15, 26] and inhibitory effect on PDGFR strengthened inhibitory effect of VEGFR to induce antitumor effects. The phenomena showing PDGF-related toxicity and efficacy may be a characteristic of TSU-68, which is dissimilar from the other multi-kinase inhibitors for anti-angiogenesis.

In the pharmacokinetics of TSU-68, the C_{max} and AUC after the repeated doses on days 2 and 28 were lower than those after the 1st dose on day 1. These parameters on day 28 were comparable with those on days 2. This suggests that the decreased plasma exposure to TSU-68 rapidly reaches a steady state and is maintained over therapeutic cycles. This trend is consistent with a published clinical result showing that AUC of TSU-68 on day 56 was similar to that on day 28 [27]. Furthermore, PK parameters of TSU-68 were not apparently different between the 55th and 56th doses (b.i.d.), and between the 82nd and 84th (t.i.d.), suggesting that a circadian rhythm had no influence on PK of TSU-68. The observed decrease in the exposure is probably due to autoinduction of TSU-68 metabolism. Since urinary excretion accounted for a very low percentage of the dose, predominant elimination of TSU-68 can be regarded as hepatic metabolism. In the non-clinical studies [28, 29], TSU-68 was found to cause induction of liver cytochrome P450, CYP1A1/2 involved in its own metabolism, leading to the decrease in the TSU-68 plasma concentrations.

No obvious dose-dependent increases were observed with C_{max} and AUC after the repeated doses. The most likely reason for this observation is a saturation of absorption based on its lower solubility. A previous clinical study reported that absolute bioavailability of TSU-68 administered at 100 mg/m^2 after meals was 42% [30]. In this study, AUC of TSU-68 administered after meals tended to be slightly higher, compared to a previous study

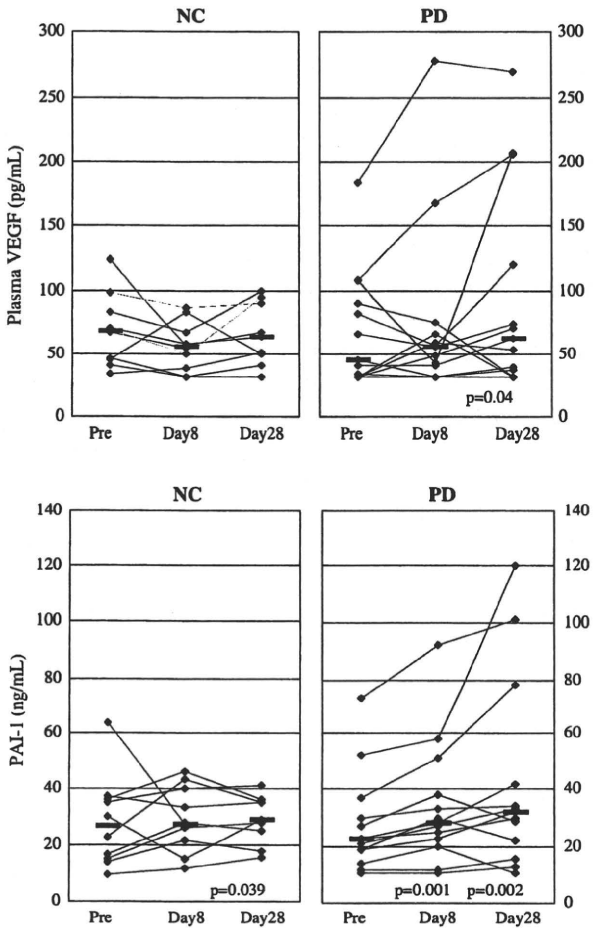

Fig. 2 The changing of plasma VEGF and PAI-1 after 8 or 28 days of treatment with TSU-68

[31], in which TSU-68 was administered between meals. Another Japanese clinical study of TSU-68 has also reported that the AUC under fed conditions was higher than that under fasted conditions [30]. These findings may suggest that absorption of TSU-68 tends to increase when food is taken just before TSU-68 dosing. When total daily AUC was compared between the b.i.d. and t.i.d. regimens, the AUC was estimated to be similar between the two regimens. Therefore, the b.i.d. regimen would be preferable in therapeutic use from the viewpoint of compliance.

As an efficacy of TSU-68, there was no tumor reduction and a few patients were treated for more than 6 months. However, the tumor control rate was 43.5% (10/23 patients). These patients had no effects with standard therapy, or did not have standard therapy previously. In light of this population, it was decided that antitumor effect not be pursued.

TSU-68 phase I studies in Japan have three different dosing regimens, including the twice-daily between meals [31] and the twice-daily or thrice-daily after meals. The recommended dosage of TSU-68 was concluded to be $\leq$800 mg/m^2 b.i.d. under the between-meal conditions, and $\leq$400 mg/m^2 b.i.d. and $\leq$400 mg/m^2 t.i.d. under the after-meal conditions. As mentioned above, AUC of TSU-68 tend to be somewhat increased by administering the drug after meals, and the total daily AUC was similar between the b.i.d. and t.i.d. regimens. In addition, marked differences in the types or severity of drug-related adverse events were not observed between the three regimens. Therefore, for further studies, the recommended dose schedule of TSU-68 administration was finally considered "b.i.d. after meals", in which higher AUC and more convenient therapeutic use would be achieved.

An Independent Data Monitoring Committee discussed data obtained from the three studies to evaluate the recommended dose of TSU-68 for further studies. The number of TSU-68 tablets administered to patients should not be adjusted by patients according to body surface area, because of no obvious dose-dependency in the steady-state AUC. Regarding safety, the incidences of drug-related adverse events showed no marked dose dependency, although the number of drug-related adverse events by patient tended to increase when patients were treated at doses of 400 mg/m^2 or more. Additionally, out of 6 patients enrolled in dose level 400 mg/m^2, which was the maximum recommended dose of TSU-68, one patient (Pt.4 in Table 1) experienced DLT (grade 3 dyspnoea, hypoxia, pleural effusion and anorexia) and one patient (Pt.5 in Table 1) experienced grade 2 anorexia which caused discontinuation of TSU-68 administration (DLT), and 2 patients were treated at a dose of 600 mg/body b.i.d. after meals. Since TSU-68 was a tablet of 200 mg, the next dose level down was 400 mg/body; both of the 2 patients (Pt.1 and Pt.2 of Table 1) in 400 mg/body b.i.d. had no DLT. Therefore, the Independent Data Monitoring Committee considered that 400 mg/body b.i.d. was more tolerable than 600 mg/body b.i.d. for safety and suggested that "400 mg/body b.i.d. after meals" was proper as the recommended dose of TSU-68.

TSU-68 is a medicine comparably safe in receptor tyrosine kinase inhibitors and is particularly useful at prolonging the survival of patients with cancer in combinations of standard chemotherapies; further combinations studies of TSU-68 with standard chemotherapy are planned in several solid tumors.

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Clinical Cancer Research

Cancer Therapy: Clinical

Phase I Dose-Escalation Study and Biomarker Analysis of E7080 in Patients with Advanced Solid Tumors

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Abstract

Purpose: E7080, an oral multitargeted receptor tyrosine kinase inhibitor, has antiangiogenic and antitumor activity. This Phase I study investigated maximum tolerated dose (MTD), dose-limiting toxicity (DLT), pharmacokinetics (PK), pharmacodynamics (PD), and efficacy in patients with advanced solid tumors.

Experimental Design: In this sequential, dose-escalation, open-label study E7080 was administered orally twice daily in a 2-week-on/1-week-off cycle. Plasma angiogenic proteins, circulating endothelial cells (CEC) and circulating progenitor cells (CEP) were measured for biomarker analysis.

Results: Twenty-seven patients (median age 53 years, performance status 0/1) were enrolled. E7080 was escalated from 0.5 to 1, 2, 4, 6, 9, 13, 16, and 20 mg bid by conventional 3-patient cohorts. During cycle 1, no grade 3/4 toxicity was observed up to 13 mg bid. DLTs included grade 3 AST/ALT increase in 1 patient at 16 mg bid and grade 3 platelet count decrease in 2 patients at 20 mg bid. The MTD of 13 mg bid was determined. After repeated doses, C_{max} and area under the plasma concentration–time curve increased in a dose-dependent manner. After 14 days' treatment, c-kit(+) CEPs and CECs significantly decreased in cycle 1, but c-kit(−) CEPs and CECs did not. Change from baseline in c-kit(+) CEC ratio in cycle 1 and baseline SDF1α, c-kit(+) CEPs and c-kit(+) CEP ratio significantly correlated with the E7080 therapeutic effect.

Conclusion: E7080 has manageable toxicity up to 13 mg bid when administered in a 2-week-on/1-week-off cycle and shows preliminary activity for durable disease control. Biomarker analysis suggested antiangiogenic activity correlated with antitumor activity in patients with a wide range of solid tumors. *Clin Cancer Res; 17(8); 2528–37. ©2011 AACR.*

Introduction

Angiogenesis, the development and proliferation of a vascular network, is fundamental to both initial tumor growth and progression to metastatic disease. VEGF is a key factor to drive tumor angiogenesis (1), and platelet-derived growth factor (PDGF) and fibroblast growth factor (FGF) also play an important role. PDGF receptor (PDGFR) tyrosine kinases are expressed on the surface of pericytes and smooth muscle cells, and both induce proliferation and contribute to vascular maturation (2, 3). FGF receptor (FGFR) tyrosine kinases expressed on the surfaces of endothelial cells (EC) and smooth muscle cells, promote signals for cell proliferation and survival, as well as the development and stabilization of blood vessels (4, 5). Upon inhibition of tumor VEGF, PDGF, and FGF may also be upregulated to induce and maintain angiogenic activity (6, 7).

The tyrosine kinase receptors for these angiogenic factors, along with their associated signaling pathways, represent putative targets for pharmacotherapeutic intervention in cancer patients. Several molecules have been developed specifically to target tyrosine kinase receptors. Multitargeted tyrosine kinase inhibitors exhibited notable antitumor effect and showed acceptable tolerability profiles (8–10). However, differences in target kinase selectivity and potency may influence individual efficacy and toxicity profiles.

E7080, an oral multitargeted receptor tyrosine kinase inhibitor with antiangiogenic and antitumor activity,

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Note: Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

This article presents original material from a Phase I study of E7080. Findings from this study have been presented at the 44th Annual Meeting American Society of Clinical Oncology, May 30 to June 3, 2008, and the 13th Workshop of the Japanese Society for Molecular Target Therapy of Cancer, June 25, 2009.

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Translational Relevance

Tyrosine kinase receptors for angiogenic factors along with their associated signaling pathways represent recognized targets for pharmacotherapeutic intervention. E7080 is an oral multitargeted receptor tyrosine kinase inhibitor that has antiangiogenic and antitumor activity, and strongly inhibits a wide range of tyrosine kinases. This Phase I dose-escalation study determined the maximum tolerated dose, dose-limiting toxicities, pharmacokinetics, pharmacodynamics, and preliminary efficacy of E7080. The correlation of certain biomarkers with antitumor activity was also evaluated. E7080 showed a manageable toxicity at 13 mg or less bid doses (only 3 DLTs at ≥16 mg bid) and preliminary activity for durable disease control. Biomarker analysis of circulating endothelial and progenitor cells, suggested an antiangiogenic activity, which correlated with antitumor activity in patients with a wide range of advanced solid tumors.

strongly inhibits a wide range of tyrosine kinases, including VEGFR-1 (Flt1), VEGFR-2 (KDR), and VEGFR-3 (Flt4), FGFR-1, PDGFRβ, and c-kit (11). E7080 decreased VEGFR-2 phosphorylation in both ECs [half maximal inhibitory concentration (IC_{50}) 0.83 nmol/L] and cell-free assays (IC_{50} 4 nmol/L; refs. 11, 12). In addition, E7080 has been shown to inhibit the growth of vascular EC and the formation of vascular-like tube structures in culture cells, and suppress tumor progression in murine models with various tumor types (11–13). Inhibition of xenograft tumor growth by E7080 was observed at doses as low as 1.0 and 10.0 mg/kg, suggesting greater efficacy of this agent compared to preapproved VEGFR2 inhibitors, including sorafenib and sunitinib (13–15).

A phase I dose-escalation study was conducted to investigate the safety, pharmacokinetics (PK), pharmacodynamics (PD; via biomarker analysis), and preliminary efficacy of E7080 in patients with advanced solid tumors. In addition, the correlation of certain biomarkers with antitumor activity was evaluated.

Patients and Methods

Study design

This single-center, open-label, sequential dose-escalation study of E7080 (ClinicalTrials.gov identifier NCT00280397; study identification number E7080-J081-103) was conducted at the National Cancer Center Hospital (Tokyo, Japan) between January 24, 2006 and September 8, 2008. All patients provided written, informed consent and study approval was obtained from the Institutional Review Board at the National Cancer Center Hospital. The study was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice Guidelines. As stipulated by Japanese guidelines, the initial starting dose of E7080 was set at the human equivalent (based on body surface area) of one third of the toxic low dose obtained in 4-week animal toxicity studies. These studies established the toxic low dose as 0.1 mg/kg, at which testicular toxicity was observed in dogs. The human equivalent dose is calculated as 3.2 mg, thus 1.0 mg was set as the initial dose for E7080 in this study.

The primary objective of the study was to determine the maximum tolerated dose (MTD) and dose-limiting toxicity (DLT) of oral E7080 administered twice daily in a 2-week-on/1-week-off cycle in patients with advanced solid tumors. Secondary objectives included the assessment of PK, safety and tolerability, as well as determining a recommended dose for Phase II trials, and describing any observed tumor responses. Exploratory objectives included the characterization of PD markers of antitumor activity.

Eligibility criteria

Patients aged 20 to 75 years with histologically or cytologically confirmed advanced solid tumors that were resistant to standard therapy, or for which no standard therapy exists, and with Eastern Cooperative Oncology Group (ECOG) performance status of 0 to 1, a life expectancy of 3 or more months, and adequate organ function were eligible. Postmenopausal women with amenorrhea for 12 or more months, or women of childbearing potential who were not pregnant, were eligible for inclusion in the study. All females and fertile male patients had to use adequate contraceptive methods during the study.

Patients were excluded if they had received previous anticancer treatments (including surgery or radiotherapy) or anticoagulant therapy (blood transfusions, blood agents, and hematopoietic factors) for at least 4 weeks prior to study entry or had incompletely recovered from prior therapy-related toxicity, except alopecia (evidence of grade ≥2 toxicity). Additional exclusion criteria included: brain metastases (symptomatic or requiring treatment); abnormal bone marrow, liver, or renal function [hemoglobin <9.0 g/dL, neutrophil count <1,500/μL, platelet count <100,000/μL, serum bilirubin >1.5 mg/dL, aspartate aminotransferase (AST) >100 IU/L, alanine aminotransferase (ALT) >100 IU/L, serum creatinine >1.5 mg/dL, or creatinine clearance <50 mL/min, measured by the Cockcroft–Gault method (16)], history of drug or alcohol abuse; infection with human immunodeficiency virus, hepatitis B or C; history of ischemic heart disease or clinically significant cardiac disorder within 6 months prior to study start; prolongation of the QT interval corrected using Fridericia's formula (QTcF) at screening (QTcF: >450 milliseconds for males and >470 milliseconds for females) or arrhythmia requiring treatment; history of cerebral infarction, hemorrhagic or thrombotic disease; evidence or history of malabsorption syndrome, surgery involving gastrointestinal anastomoses 4 or less weeks prior to enrollment or were recovering from surgery within 3 weeks of enrollment. Other exclusions included patients with duplicate resting mean systolic blood pressure ≥160 mmHg and diastolic blood pressure

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≥90 mmHg measurements or evidence of proteinuria at screening, those taking antiplatelet/anticoagulant therapy at screening and throughout the study, and patients receiving any other investigational drug within 4 weeks prior to study entry. Prophylactic administration of drugs including antiemetics, antihypertensives, and antidiarrheal agents was prohibited during Cycles 0 and 1. Concomitant use of cytochrome P450 (CYP3A4) inhibitors or inducers was prohibited throughout the study, due to potential interactions with or effects on metabolism of E7080.

Study treatment

Eligible patients were sequentially enrolled on escalating doses of oral E7080 using a standard 3 + 3 design. Dosing was scheduled to begin at 0.5 mg bid. In cycle 0 (7-day cycle), patients received a single oral dose of E7080 on day 1 for PK analysis and received no drug on days 2 to 7. In cycle 1 (21-day cycle), which immediately proceeded cycle 0, patients received E7080 bid on days 1 to 14. All patients were hospitalized for E7080 administration and evaluation during the full 28 days of cycles 0 and 1, thereafter the study was continued on an outpatient basis.

After tolerability was confirmed in cycles 0 and 1, the dose was doubled if a hematologic toxicity (≤grade 1 including anemia or lymphocytopenia) or nonhematologic toxicity (excluding alopecia and hypertension) in Cycle 1 was observed. When grade 2 toxicity occurred in 1 or more patient, the dose was escalated by 50% or less and, if grade 3 toxicity occurred, the dose was escalated by 33.3% or less thereafter.

Before dose escalation, all 3 patients in each cohort were required to complete cycle 1 of treatment. If no patients experienced a DLT at the first dose level, then the dose was escalated for the next 3 patients. If 1 of these experienced DLT, then 3 more patients were accrued at the same dose level. If none of these additional patients experienced a DLT, then the dose was escalated for the subsequent 3 patients. Dose escalation was terminated when 2 or more patients experienced a DLT at a given dose level. No intrapatient dose escalations were allowed. The presence of DLTs was assessed during cycles 0 and 1. From cycle 2 onwards, patients remained on study treatment at the same dose level as cycle 1 until tumor progression, unacceptable toxicity, or withdrawal due to other reasons.

Dose delays and reductions. To allow a patient to recover from any toxicities, a treatment cycle could be delayed for 14 or less days. Any patient who experienced a DLT that resolved sufficiently to allow continued treatment was eligible for treatment at a reduced dose level (≤75% and ≤50% of the previous dose for the first and second dose reductions, respectively). A maximum of up to 2 dose reductions was permitted.

Safety assessments

DLTs and MTD. DLTs were defined as grade 3 or more platelet count decrease, grade 4 neutropenia, any grade 3 or more nonhematologic toxicity (with exceptions of grade 4 hypertension not controlled by any antihypertensive drugs and grade ≥3 vomiting and diarrhea not controlled by antiemetic or antidiarrheal drugs), and failure to administer more than 75% of the planned doses of E7080 during the same cycle due to toxicity.

The MTD was defined as the highest dose at which no DLT was experienced by the first 3 patients in that cohort, or the dose at which a DLT was experienced by no more than 1 of 6 patients evaluable for toxicity.

Laboratory assessments and adverse events

Safety assessments scheduled for screening, throughout the study, and on study discontinuation included medical history, ECOG performance status, physical examination, vital signs, laboratory tests (hematology, blood biochemistry, and blood coagulation), urinalysis, electrocardiogram, and pregnancy testing. Adverse events (AE), including DLTs, were assessed throughout the study according to the Common Terminology Criteria for AEs (CTCAE Version 3.0; ref. 17).

Pharmacokinetics

In cycle 0, patients received a single oral dose of E7080 for PK analysis. Blood samples were collected at predose on day 1 and at 1, 3, 5, 6, 8, 12, 24, 48, 96, and 168 hours following administration. In cycle 1, patients received E7080 twice daily on days 1 to 14 of a 21-day cycle, except day 14 when E7080 was administered only once in the morning for PK analysis. Blood samples were collected from each patient before the first dose on days 1, 5, 8, 11, and 14 and at 1, 3, 5, 6, 8, 12, 24, 48, 96, and 168 hours after administration on day 14. Urine samples were collected 0 to 12 hours (the time equivalent to the interval between doses) after administration on day 14 in cycle 1. Plasma and urine E7080 concentrations were determined using liquid chromatography with tandem-mass spectrometry (Sumitomo Chemical Co. Ltd.).

Antitumor activity

Best overall tumor response and disease progression were measured using the Response Evaluation Criteria in Solid Tumors (RECIST; ref. 18). Tumor assessments were evaluated at screening, in cycles 2 and 3, and in every 2 cycles thereafter.

PD and baseline biomarkers

Blood samples for PD analysis were collected from each patient at predose of day 1 and 15 in cycle 1. Circulating endothelial cells (CEC) and circulating progenitor cells (CEP), which reflect active vascular turnover and angiogenesis (19, 20) were collected and measured within 24 hours of blood collection by fluorescence activated cell sorting (FACS). Briefly, peripheral blood mononuclear cells were incubated for 30 minutes at 4°C with fluorescein isothiocyanate (FITC)-conjugated anti-human CD34, FITC-conjugated anti-human CD45, phyroerythrin-conjugated anti-human CD117 (c-kit), and with FITC-conjugated anti-human CD133. Cells were then washed with phosphate-buffered saline and fixed in 4% paraformaldehyde,

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prior to FACS analysis, performed by SRL MediSearch Inc. using a FACScan cytometer and CellQuest software (Becton Dickinson).

To quantify CEC and CEP, the number of CD34-positive and CD45-negative cells was isolated, and CD133-negative cells and CD133-positive cells were determined as CEC and CEP, respectively. In addition, CEC and CEP were divided into c-kit positive [c-kit(+)] and negative [c-kit(−)] subpopulations. C-kit(+) ratio (%) was calculated as [c-kit(+) CEC or CEP]/[total CEC or CEP].

Plasma samples were collected before the first dose and stored at −80°C until assayed. Samples were analyzed in triplicate for baseline levels of angiogenic proteins and cytokines using a BioPlex (Bio-Rad Laboratories, Inc) assay (Mitsubishi Chemical Medience Corp.; ref. 21). Soluble VEGFR-1 (sVEGFR-1) and soluble VEGFR-2 (sVEGFR2) were measured by enzyme-linked immunosorbent assay (22).

Correlations of biomarker levels with the therapeutic effect of E7080 were investigated. Therapeutic effect was defined as the treatment duration from the first E7080 dose to discontinuation due to progressive disease or toxicity.

Statistical analysis

All patients who received at least 1 E7080 dose and had evaluable data were included in the safety, efficacy, PK, and PD analyses. PK analysis of plasma E7080 concentration-versus-time data were analyzed using WinNonlin Version 5.2 software. Noncompartmental analysis was performed to determine PK parameters of E7080. PD analysis was performed using Spearman's rank correlation coefficient for correlation analysis and Wilcoxon signed rank test to determine change from pretreatment.

Results

Patient characteristics

Twenty-seven evaluable patients received E7080. Demographic and baseline characteristics of these patients are shown in Table 1. Patients with a wide range of solid tumors were enrolled, with colon cancer being the most frequent (33.3%). The majority of patients (81.4%) had received 2 or more prior chemotherapy regimens.

Study treatment

Of the 27 patients who received E7080, 26 patients completed at least cycle 1, and 10 patients continued treatment for ≥6 cycles. One patient who received 6 mg bid did not complete cycle 0 due to a postrenal failure AE (not a DLT) and was excluded from the efficacy and PD populations. Across all dose groups, the main reason for study withdrawal in patients who completed at least cycle 1 was progressive disease (20/26 patients). Other reasons for withdrawal were AEs ($n = 2$), start of treatment in next cycle delayed ≥15 days ($n = 2$), withdrawal of consent ($n = 1$), and investigator decision ($n = 1$).

Table 1. Patient characteristics (treated patients, $N = 27$)

Characteristic	
Mean age, y (range)	50.7 (26–70)
Gender, *n* (%)	
Male	10 (37.0)
Female	17 (63.0)
ECOG performance status, *n* (%)	
0	10 (37.0)
1	17 (63.0)
2	0 (0)
Mean time since initial diagnosis, months (range)	46.04 (9.7–120.1)
Site of primary lesion, *n* (%)	
Colon cancer	9 (33.3)
Sarcoma	7 (25.9)
Non–small cell lung cancer	5 (18.5)
Other	6 (22.2)
Histologic/cytologic diagnosis, *n* (%)	
Adenocarcinoma	15 (55.6)
Squamous cell carcinoma	3 (11.1)
Bone or soft-tissue carcinoma	7 (25.9)
Other	2 (7.4)
Prior treatment history, *n* (%)	
Surgery	25 (92.6)
Radiotherapy	6 (22.2)
Chemotherapy	26 (96.3)
Number of prior chemotherapy regimens, *n* (%)	
0	1 (3.7)
1	4 (14.8)
2	3 (11.1)
3	9 (33.3)
≥4	10 (37.0)

Safety

DLTs and MTD. No DLTs were observed during cycle 0 and 1 of the dose escalation at 0.5, 1, 2, 4, 6, 9, and 13 mg bid dose levels. DLTs were reported in 2 patients at 20 mg bid, both of whom experienced grade 3 platelet count decrease. Consequently, 3 patients were accrued at the 16 mg bid dose, 1 of whom developed DLT (increased grade 3 AST and ALT). Of the other 2 patients in the 16 mg bid group, 1 developed grade 2 platelet count decrease in cycle 1 and grade 2 fatigue in cycle 2, while the other patient experienced grade 3 fatigue, grade 3 proteinuria, and grade 2 edema in cycle 2. No additional patients were treated at the 16 mg bid dose level as it was judged to be an intolerable dose. Based on the DLTs observed, the MTD was defined as 13 mg bid for this dosing schedule.

Adverse events

The most frequently reported AEs (≥50% of patients) were: hematuria (74.1%), fatigue (70.4%), hypertension

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Table 2. Summary of AEs (≥20% all grades, all cycles; $n = 27$)

AEs	Patients, *n* (%)				
	Grade 1	Grade 2	Grade 3	Grade 4	Total
Hematuria	20 (74.1)	0	0	0	20 (74.1)
Fatigue	13 (48.1)	5 (18.5)	1 (3.7)	0	19 (70.4)
Hypertension	0	13 (48.1)	5 (18.5)	0	18 (66.7)
AST increased	12 (44.4)	2 (7.4)	3 (11.1)	0	17 (63.0)
Headache	17 (63.0)	0	0	0	17 (63.0)
Proteinuria	5 (18.5)	10 (37.0)	2 (7.4)	0	17 (63.0)
ALT increased	10 (37.0)	3 (11.1)	2 (7.4)	0	15 (55.5)
Diarrhea	9 (33.3)	4 (14.8)	2 (7.4)	0	15 (55.5)
Blood LDH increased	10 (37.0)	2 (7.4)	2 (7.4)	0	14 (51.9)
Blood albumin decreased	8 (29.6)	5 (18.5)	0	0	13 (48.1)
Blood alkaline phosphatase increased	11 (40.7)	1 (3.7)	1 (3.7)	0	13 (48.1)
Anorexia	7 (25.9)	4 (14.8)	1 (3.7)	0	12 (44.4)
Nausea	10 (37.0)	1 (3.7)	1 (3.7)	0	12 (44.4)
GGT increased	3 (11.1)	6 (22.2)	2 (7.4)	0	11 (40.7)
Platelet count decreased	5 (18.5)	3 (11.1)	3 (11.1)	0	11 (40.7)
Blood fibrinogen increased	10 (37.0)	0	0	0	10 (37.0)
Odema peripheral	9 (33.3)	1 (3.7)	0	0	10 (37.0)
Nasopharyngitis	9 (33.3)	0	0	0	9 (33.3)
Protein total decreased	9 (33.3)	0	0	0	9 (33.3)
Vomiting	5 (18.5)	2 (7.4)	1 (3.7)	0	8 (29.6)
Blood creatinine decreased	5 (18.5)	2 (7.4)	0	0	7 (25.9)
Blood TSH increased	6 (22.2)	1 (3.7)	0	0	7 (25.9)
Blood urea increased	7 (25.9)	0	0	0	7 (25.9)
Constipation	7 (25.9)	0	0	0	7 (25.9)
Dyspnea	5 (18.5)	0	2 (7.4)		7 (25.9)
WBC count increased	3 (11.1)	4 (14.8)	0	0	7 (25.9)
Anemia	3 (11.1)	1 (3.7)	2 (7.4)	0	6 (22.2)
Dizziness	6 (22.2)	0	0	0	6 (22.2)
Hyperlipidemia	1 (3.7)	4 (14.8)	1 (3.7)	0	6 (22.2)

Abbreviations: GGT, γ-glutamyltransferase; TSH, thyroid stimulating hormone; WBC, white blood cells.

(66.7%), AST increased (63.0%), headache (63.0%), proteinuria (63.0%), ALT increased (55.5%), diarrhea (55.5%), and lactate dehydrogenase (LDH) increased (51.9%; Table 2).

Five patients experienced 6 serious AEs (SAEs) considered to be related or possibly related to study medication, which included hypertension (0.5 and 6 mg bid), hemorrhage (6 mg bid), pneumonia and worsening dyspnea (9 mg bid) and platelet count decrease (9 mg bid).

In total, 27 dose reductions were recorded, 3 each at 0.5, 1, 2, 4, 9, and 13 mg bid doses and 4 at the 6 mg dose. One patient who received 6 mg bid discontinued the study due to postrenal failure AE. One patient died due to worsening underlying disease during the study.

Pharmacokinetics

All patients had measurable plasma E7080 concentrations (>0.08 ng/mL) up to 168 hours after administration of either a single oral E7080 dose, or after 14 days of twice daily E7080 administration. Although the concentration was below the limit of quantification in 5 samples at 168 hours after single last dose, they did not affect the overall analysis. Maximal plasma concentration (C_{max}), the time to peak plasma concentration (t_{max}) and elimination half-life ($t_{1/2}$) for E7080 after a single dose and during steady state (ss) were similar (Table 3). C_{ssmax} and area under the plasma concentration–time curve (AUC) from time zero to the last measurable concentration ($AUC_{0-\tau}$) were dose proportional (Fig. 1).

The serum protein binding rates ranged from 96.6% to 98.2%. The previously reported IC_{50} of E7080 for VEGFR-2 phosphorylation in EC was 0.83 nmol/L (11), which based on 96.6% to 98.2% of E7080 being protein bound is approximately equivalent to a plasma concentration of 17 ng/mL. The IC_{50} of E7080 in plasma was almost equivalent to a maximum plasma concentration (C_{max}) at 0.5 mg bid and to a minimum plasma concentration (C_{min}) at 2 mg bid in multiple dosing (Table 2). After

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Table 3. Pharmacokinetic parameters for E7080 following single administration on day 1 of a 21-day cycle (cycle 0) and twice daily on days 1 to 14[a] of a 21-day cycle (cycle 1)

Parameter[b]	E7080 dose levels, mg bid									
	0.5	1	2	4	6	9	13	16	20[c]	
Cycle 0 (single dose)[d]										
n	3	3	3	3	4	3	3	3	2	
C_{max}, ng/mL	2.5 (0.2)	5.3 (2.5)	18.4 (3.5)	61.3 (25.6)	99.3 (20.6)	201.4 (49.4)	302.7 (72.5)	471.5 (151.7)	329.2	674.2
t_{max}, h	5 (3–5)	3 (3–5)	3 (1–3)	1 (1–3)	3 (1–3)	1 (1–3)	1 (1–3)	1 (1–1)	3	3
AUC_{0-24}, ng h/mL	41 (2.0)	75 (30)	218 (33)	511 (111)	876 (165)	1,329 (379)	2,319 (339)	3,047 (597)	2,270	4,751
$AUC_{0-\infty}$, ng h/mL	115 (27)	164 (76)	429 (89)	759 (89)	1,202 (265)	1,658 (460)	2,744 (418)	3,419 (515)	2,849	5,405
$t_{½}$, h	46.5 (5.9)	30.3 (8.9)	36.4 (4.0)	32.0 (5.9)	31.6 (5.0)	28.6 (4.0)	25.0 (8.2)	19.1 (13.0)	38.1	31.6
Cycle 1 (multiple dosing)[e]										
n	3	3	3	3	3	3	3	3	1	
C_{ssmax}, ng/mL	16.7 (5.2)	23.7 (9.4)	68.6 (23.3)	154.0 (33.8)	178.2 (38.0)	384.4 (168.5)	556.8 (108.7)	713.3 (276.8)	393.5	–
C_{ssmin}, ng/mL	7.2 (2.6)	9.5 (4.6)	20.3 (3.8)	39.6 (7.7)	57.1 (21.4)	74.4 (32.6)	138.3 (40.1)	149.4 (52.5)	85.0	–
t_{maxss}, h	1 (1–3)	3 (3–3)	1 (1–3)	3 (1–3)	3 (1–6)	1 (1–1)	3 (1–3)	3 (1–3)	3	–
$AUC_{0-\tau}$, ng·h/mL	128 (36)	198 (86)	483 (117)	1,022 (246)	1,186 (141)	2,169 (803)	3,824 (622)	4,228 (1,485)	2,519	–
$t_{½ss}$, h	37.1 (1.0)	32.7 (3.4)	36.3 (6.4)	36.3 (1.7)	32.6 (2.5)	32.8 (2.7)	32.6 (6.8)	25.6 (7.1)	38.5	–

[a]E7080 administered only in the morning of day 14, cycle 1.
[b]Data are shown as mean (SD), except for t_{max} and t_{maxss} which are median (range).
[c]For E7080 20 mg bid, individual values are displayed for each patient.
[d]The pharmacokinetic profile was evaluated in cycle 0 after single dosing in 26 patients.
[e]The pharmacokinetic profile was evaluated in cycle 1 after multiple dosing in 25 patients.

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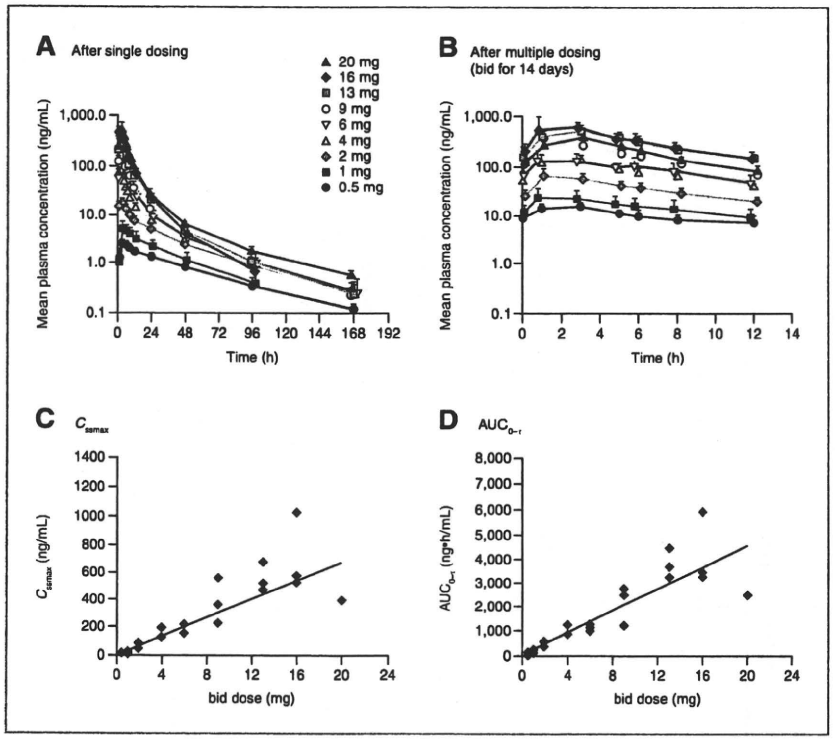

Figure 1. Pharmacokinetic profile of E7080. Plasma concentration-time profiles (A) after single dosing and (B) after multiple dosing. Data shown as mean + SD. Dose-dependent increase of (C) C_{max} and (D) $AUC_{0-\tau}$ at ss after multiple dosing.

repeated E7080 administration, urinary excretion of the parent compound ($fe_{0-\tau}$) ranged from 0.5% to 2.0%, and renal clearance was 17.4 to 84.6 mL/h, with no uniform trends observed across the dose range studied.

Antitumor activity

In 9 dose cohorts ranging from 0.5 to 20 mg bid, 27 patients received E7080 treatment for a median of 4.0 cycles (range 1–12). The median treatment duration was 86.0 days (range 1–270). Treatment duration was independent of E7080 dose level. Of 26 patients in the efficacy population, 25 were evaluable for response by RECIST. A partial response was documented in 1 patient with colon cancer at cycle 4 of E7080 2 mg bid which continued until cycle 10, when progressive disease was reported. Stable disease was recorded as best overall response in 21 patients, 84% of the evaluable patients.

Pharmacodynamics

Change in CEP and CEC number and correlation with E7080 therapeutic effect. The total number of CEPs decreased after 14 days' treatment with E7080 ($P < 0.001$). However, only the number of c-kit(+) CEPs decreased significantly ($P < 0.001$), and c-kit(−) CEP number was not affected (Fig. 2A). In contrast, while no change was seen in the total number of CECs, c-kit(+) CECs decreased significantly ($P < 0.01$) and c-kit(−) CECs increased significantly ($P < 0.001$; Fig. 2B). The c-kit(+) ratio in both CEP and CEC populations decreased upon E7080 treatment (Fig. 2C, D), although this was independent of E7080 dose (Fig. 2E, F). The reduction in c-kit(+) ratio in CECs associated with E7080 treatment correlated with treatment duration (Spearman's rank correlation coefficient $\rho = -0.468$; $P = 0.018$), while no correlation of c-kit(+) ratio in CEPs with treatment duration was observed (Fig. 2G, H).

Correlation of baseline biomarker levels with E7080 therapeutic effect

Significant inverse correlations were observed with E7080 treatment duration and baseline levels of c-kit(+) CEP and c-kit(+) ratio in CEP, but not CEC (Supplementary Table SA1). Similarly, analysis of baseline levels of angiogenic proteins and cytokines, including key CEP and CEC regulatory factors, revealed a significant inverse correlation with E7080 treatment duration and predose levels of plasma SDF1α (Supplementary Table SA2). These data suggest that patients with higher baseline levels of these biomarkers showed shorter treatment duration.

Discussion

In this Phase I dose escalation study, PK, PD, and preliminary efficacy of E7080 was investigated in patients

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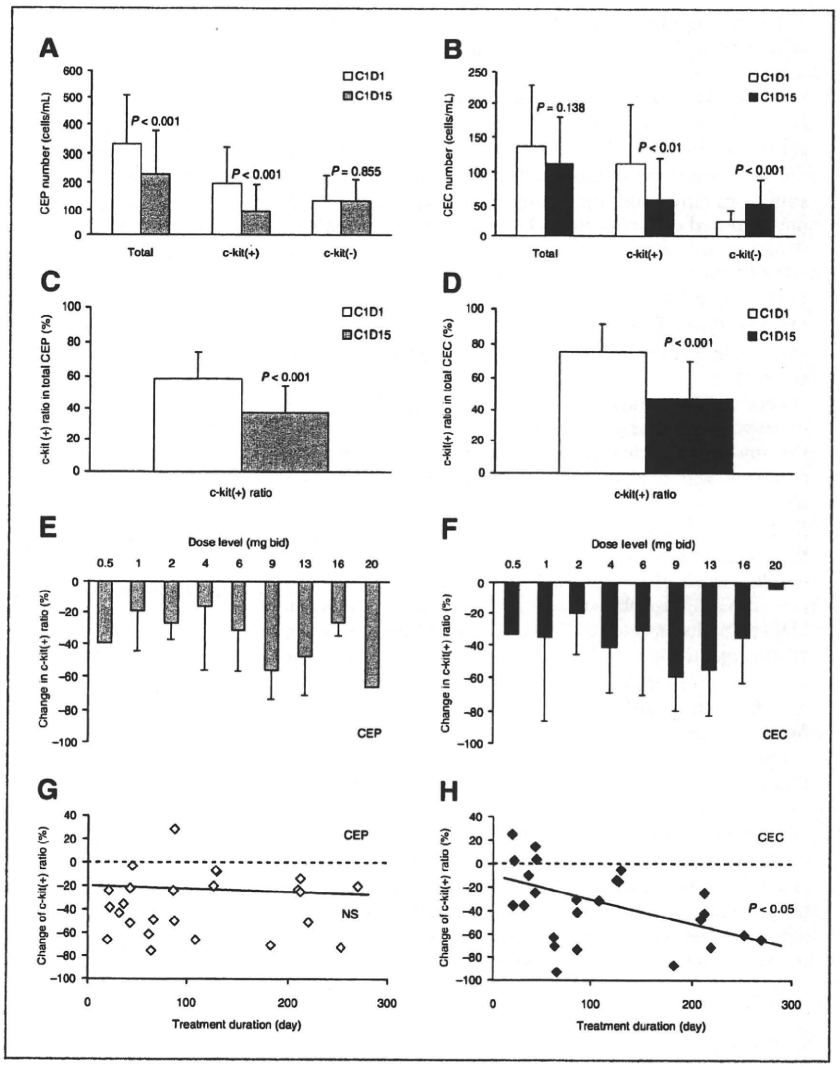

Figure 2. Decrease of CEP and CEC number associated with E7080 and correlation with treatment duration. A, 14-day E7080 treatment decreased total CEP, c-kit CEP, but not c-kit(−) CEP. B, E7080 treatment did not affect total CEC number, but decreased c-kit(+) CECs, and increased c-kit(−) CECs. C and D, E7080 decreased c-kit(+) ratio in CEP and CEC populations, respectively. Change in CEC and CEP number from cycle 1 day 1 (C1D1) to day 15 (C1D15) were statistically analyzed for each patient by Wilcoxon signed rank test. E and F, the decrease of c-kit(+) ratio was independent of E7080 dose level in CEP and CEC populations. G and H, the decrease in c-kit(+) ratio associated with E7080 correlated with treatment duration for CECs but not for CEPs. NS, not significant.

with advanced solid tumors. E7080 demonstrated a manageable toxicity profile at doses of 0.5 to 13 mg bid. Only 3 DLTs were reported, all with E7080 doses of 16 mg or more bid. Based on the occurrence of 1 DLT or more in the E7080 16 and 20 mg bid groups, 13 mg bid was considered to be the MTD when E7080 was administered in a 2-week-on/1-week-off cycle. The PK parameters of E7080, after repeated doses, were dose proportional within the dose range of 0.5 to 20 mg bid. The elimination half-life during ss was approximately 30 hours.

The previously reported IC_{50} of E7080 for VEGFR-2 phosphorylation in EC was 0.83 nmol/L (11), which is approximately equivalent to a plasma concentration of 17 ng/mL on the basis of 96.6% to 98.2% of E7080 being protein bound. The C_{min} reached the IC_{50} and the C_{max} was 4-fold higher than the IC_{50} at 2 mg bid. These data suggest that E7080 may suppress VEGFR-2 activity at doses of 2 mg or more bid during multiple dosing.

As reported in another clinical study of E7080 (23), hypertension and proteinuria were induced frequently (Table 2). These effects have been documented upon administration of several inhibitors of the VEGF signaling pathway, such as bevacuzimab and cediranib (24, 25), due to a possible perturbation of endothelial cell function (23). In this present study, hypertension was well managed by antihypertensive agents and proteinuria was managed by dose reductions or delays, and did not cause dose interruptions at the MTD or lower doses.

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The subpopulations of CEC and CEP may be predictive of disease or clinical responsiveness to anti-VEGF agents to a greater extent (26). E7080 has previously been shown to decrease the number of total CEC in tumor-bearing mice (11). In the study presented here, E7080 reduced the subpopulations of CEP and CEC that express c-kit, but did not reduce the number cells negative for c-kit expression from either subpopulation. C-kit and its ligand SCF are expressed on activated EC layers and play a key role in the survival and differentiation of cultured EC and in CEP recruitment during tumor angiogenesis (27, 28). E7080 may suppress the production of c-kit(+) CEP in bone marrow through inhibition of c-kit kinase, which may contribute to the antitumorigenic effects observed in this study (11).

Levels of biomarkers at baseline may be useful predictors of response and assist in selecting the most appropriate therapy for individual patients. Higher baseline CEC was correlated with delayed disease progression in patients with non–small cell lung and breast cancer (29, 30). We did not find a correlation between baseline CEC numbers and therapeutic effect, however significant correlations between baseline levels of SDF1, c-kit(+) CEP number and c-kit(+) ratio in CEC were shown with E7080 treatment duration. SDF1α and its receptor CXCR4 enhance CEP accumulation at angiogenic sites and are important in antiangiogenic therapy resistance (31, 32). Therefore, a high baseline level of SDF1α and c-kit(+) CEP may be a possible biomarker for predicting tumor resistance to E7080 treatment.

Dosing schedules of E7080 were evaluated in 2 other Phase I studies and a recommendation of 25 mg once daily or 10 mg bid without treatment-off period was made (33, 34). These studies also reported DLTs of grade 2 or less proteinuria and hypertension, as well as low incidences of grade 3/4 hemorrhage and thrombosis, tachycardia and fatigue (33, 34). Recent analysis has indicated that no difference between qd and bid regimen is observed with respect to exposure safety and efficacy (35). However, E7080 at 25 mg qd was recommended for future studies as this dose allows the targeting of higher exposures compared to 10 mg bid (35). A number of Phase II studies are currently recruiting or underway and the most common dosing regimen employed is 24 mg qd, although several studies are being initiated with dose-finding Phase I trials (NCT00784303, NCT01111461, NCT01136967, NCT01137604, NCT01133756, NCT00946153, NCT01133977, NCT01136733; www.clinicaltrials.gov).

In conclusion, this Phase I study has shown that E7080 was generally well tolerated and determined the MTD as 13 mg bid when administered in a 2-week-on/1-week-off cycle. Biomarker analyses suggest an antiangiogenic activity correlated with therapeutic effect in patients with a wide range of solid tumors. Studies are warranted to continue the evaluation of E7080 clinical efficacy and safety.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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