Table 3. Most Common Grade 3 to 5 Adverse Events and Adverse Events of Special Interest to Bevacizumab (related and unrelated events; safety population)

	Fluoropyrimidine-Cisplatin +			
	Bevacizumab (n = 386)		Placebo (n = 381)	
MedDRA Term	No.	%	No.	%
Any adverse events	293	76	293	77
Neutropenia	136	35	140	37
Febrile neutropenia	18	5	16	4
Anemia	40	10	53	14
Decreased appetite	32	8	41	11
Nausea	27	7	39	10
Vomiting	24	6	34	9
Diarrhea	32	8	17	4
Hypokalemia	13	3	21	6
Hand-foot syndrome	25	6	13	3
Pulmonary embolism	12	3	18	5
Bevacizumab special adverse events				
Any special adverse event	76	20	56	15
Venous thromboembolic event	25	6	36	9
Arterial thromboembolic event	5	1	8	2
Hypertension	24	6	2	< 1
Hemorrhage	9	2	9	2
GI perforation	9	2	1	< 1
Congestive heart failure	2	< 1	1	< 1
Proteinura	2	< 1	0	0
Wound complications	2	< 1	0	0
Reversible posterior leukoencephalopathy syndrome	1	< 1	0	0
Fistula/abscess	0	0	0	0

Abbreviation: MedDRA, Medical Dictionary for Regulatory Affairs, version 12.1.

survived as expected (median OS, 10.0 months), consistent with the capecitabine-cisplatin arms of previous studies.[9,21]

Preplanned subgroup analyses in AVAGAST suggest regional differences in the efficacy of antiangiogenic therapy. Patients enrolled in North America and Latin America appeared to have a survival benefit with the addition of bevacizumab (median, 11.5 *v* 6.8 months for placebo-chemotherapy; HR, 0.63; 95% CI, 0.43 to 0.94), whereas patients enrolled in Asia (90% from Japan and

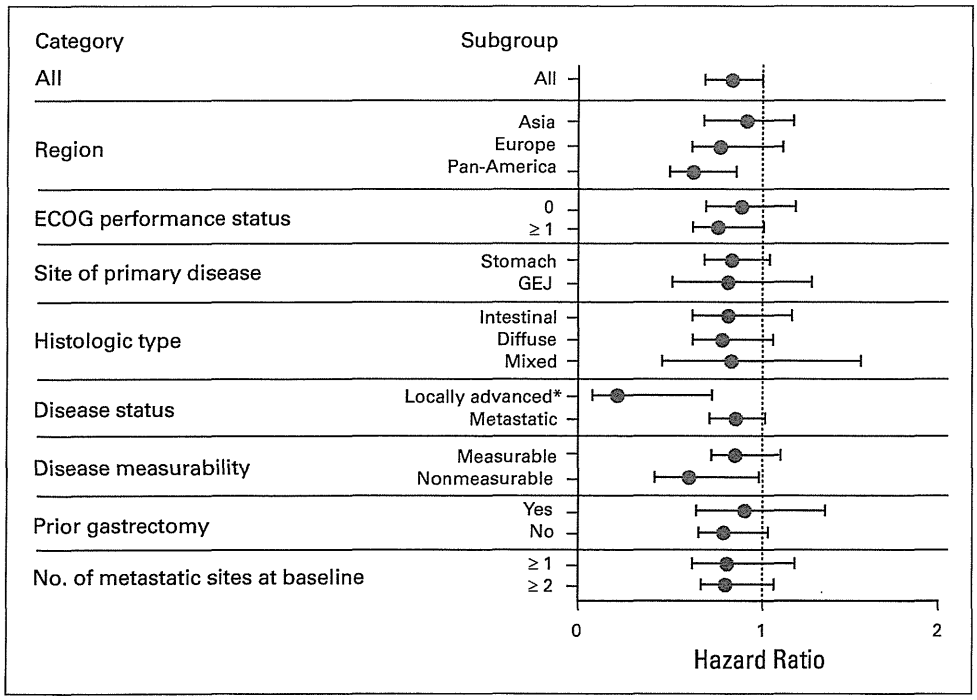

Fig 3. Subgroup analysis of overall survival in the intent-to-treat population. (*) Twenty-nine patients with locally advanced disease only. ECOG, Eastern Cooperative Oncology Group; GEJ, gastroesophageal junction.

Table 4. Baseline Characteristics and Efficacy by Region (intention-to-treat population)

	Asia							Europe							Pan-America						
	Fluoropyrimidine-Cisplatin +							Fluoropyrimidine-Cisplatin +							Fluoropyrimidine-Cisplatin +						
	Bevacizumab (n = 188)		Placebo (n = 188)					Bevacizumab (n = 125)		Placebo (n = 124)					Bevacizumab (n = 74)		Placebo (n = 75)				
Characteristic	No.	%	No.	%	HR	OR	95% CI	No.	%	No.	%	HR	OR	95% CI	No.	%	No.	%	HR	OR	95% CI
Sex																					
Male	68		67					66		67					64		65				
Female	32		33					34		33					36		35				
Age, years																					
Median	58.5		59.0					59.0		59.0					53.5		56.0				
Range	27-78		27-78					31-81		28-80					22-77		22-82				
ECOG performance status																					
0-1	98		95					89		93					95		97				
≥ 2	2		5					11		7					5		3				
Primary tumor site																					
Stomach	93		95					77		78					85		83				
Gastroesophageal junction	7		5					23		22					15		17				
Measurable disease	76		70					87		89					81		73				
Tumor histology (Lauren's classification)																					
Intestinal	36		27					49		50					35		31				
Diffuse	53		61					31		38					50		59				
Mixed	7		5					11		10					11		7				
Missing	4		7					9		2					4		4				
Liver metastases	29		26					35		38					42		41				
Prior gastrectomy	32		31					22		25					31		23				
Poststudy therapies																					
Patients with at least one treatment	59		67					24		29					24		15				
Efficacy																					
Median overall survival, months	13.9		12.1		0.97		0.75 to 1.25	11.1		8.6		0.85		0.63 to 1.14	11.5		6.8		0.63		0.43 to 0.94
Median progression-free survival, months	6.7		5.6		0.92		0.74 to 1.14	6.9		4.4		0.71		0.54 to 0.93	5.9		4.4		0.65		0.46 to 0.93
Response rate	142		132					109		110					60		55				
Overall response rate	47.9		45.5			1.10	0.69 to 1.77	41.3		28.2			1.79	1.02 to 3.15	50.0		36.4			1.75	0.83 to 3.69

Abbreviations: ECOG, Eastern Cooperative Oncology Group; HR, hazard ratio; OR, odds ratio.

Korea) appeared to have no benefit (HR, 0.97; 95% CI, 0.75 to 1.25), and European patients had intermediate results (HR, 0.85; 95% CI, 0.63 to 1.14). Differences in median OS in the comparator arm, broken down by region, were also observed (median OS, 12.1 months, Asia; 8.6 months, Europe; 6.8 months, Pan-America). These estimates are robust because 60% to 70% of patients from each region had died at the time of analysis.

Although gastric cancer is a global disease, it is not uniform. There are differences in the presentation and management of gastric cancer patients in different countries and regions. Specifically, as observed in AVAGAST, Asian patients with advanced gastric cancer more commonly receive second and further lines of therapy, more frequently have a prior history of gastrectomy, present with a higher proportion of nonmeasurable disease, and have liver metastases or proximal or gastroesophageal junction tumors less frequently. There are imbalances in the histologic tumor types across the geographic regions and between the treatment arms within the respective regions. Differences in independent prognostic factors and use of subsequent therapies may explain the different OS results between geographic regions. However, whether differences in the spectrum of gastric cancer between regions and/or different treatment practices are responsible for the regional differences for PFS in AVAGAST remains an unanswered question. An additional question is whether bevacizumab dose influences efficacy, because previous phase II studies of bevacizumab in gastric cancer incorporated a higher dose (10 or 15 mg/kg)[20,27,28] than AVAGAST (7.5 mg/kg). Further investigations are needed to answer these questions.

AVAGAST showed that adding bevacizumab to fluoropyrimidine-cisplatin did not substantially alter the safety profile of the chemotherapy backbone. Sixty-day mortality was less in the bevacizumab group (3% *v* 6% with placebo) and compares well with results from other pivotal trials in metastatic cancer of the gastric tract.[16,29] The safety profile of bevacizumab in this study was also consistent with that of previous trials in patients with other solid tumors. No new safety signals were observed. The incidence of GI perforation with

bevacizumab in AVAGAST was low (2%) and in keeping with incidences observed with bevacizumab in other cancers (0% to 2.5%)[16-19,29,30] and with chemotherapy.[31] In this study, we did not observe an increase in other bevacizumab-related toxicities, in particular venous or arterial thromboembolic events or bleeding. We also confirm the safety of a shorter bevacizumab infusion time (ie, 30 minutes and then 15 minutes for subsequent doses).[32]

Overall, these findings are indicative of clinical activity of bevacizumab plus chemotherapy as first-line therapy in advanced gastric cancer. Several lessons have been learned. It is possible that improved (and significant) bevacizumab efficacy may be achieved by refining the selection of the patient population. The ongoing biomarker analyses from this study may also help identify a subgroup of patients that preferentially benefit from the addition of an antiangiogenic agent.

In conclusion, although this trial did not reach its primary objective, the addition of bevacizumab to chemotherapy was associated with significant increases in PFS and overall response rate in the first-line treatment of advanced gastric cancer. Regional analysis indicated increased benefit in the European and Pan-American regions. Further analysis, particularly with data from the AVAGAST biomarker program, may lead to a better understanding of the study outcome, which patient subgroups will benefit from bevacizumab treatment, and how best to design future trials with bevacizumab in metastatic gastric cancer.

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Cancer Therapy: Clinical

Clinical Cancer Research

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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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

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
$t_{1/2}$, 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	–
$t_{1/2ss}$, 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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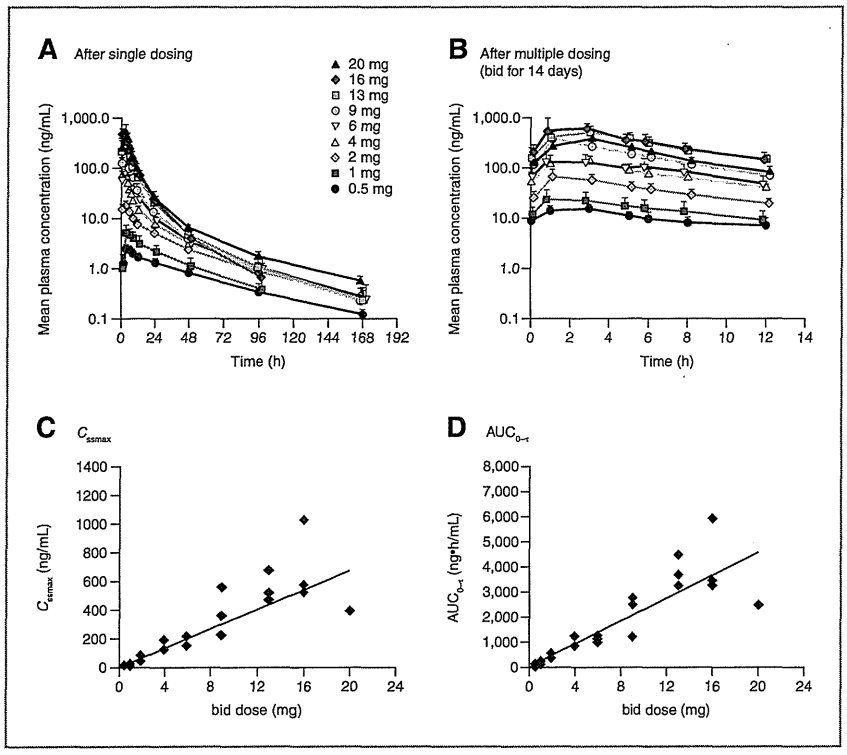

Figure 1. Pharmacokinetic profile of E7080. Plasma concentration-time profiles (A) after single dosing and (B) after multiple dosing. Data shown as mean + SD. Dose-dependent increase of (C) C_{max} and (D) $AUC_{0-\tau}$ at ss after multiple dosing.

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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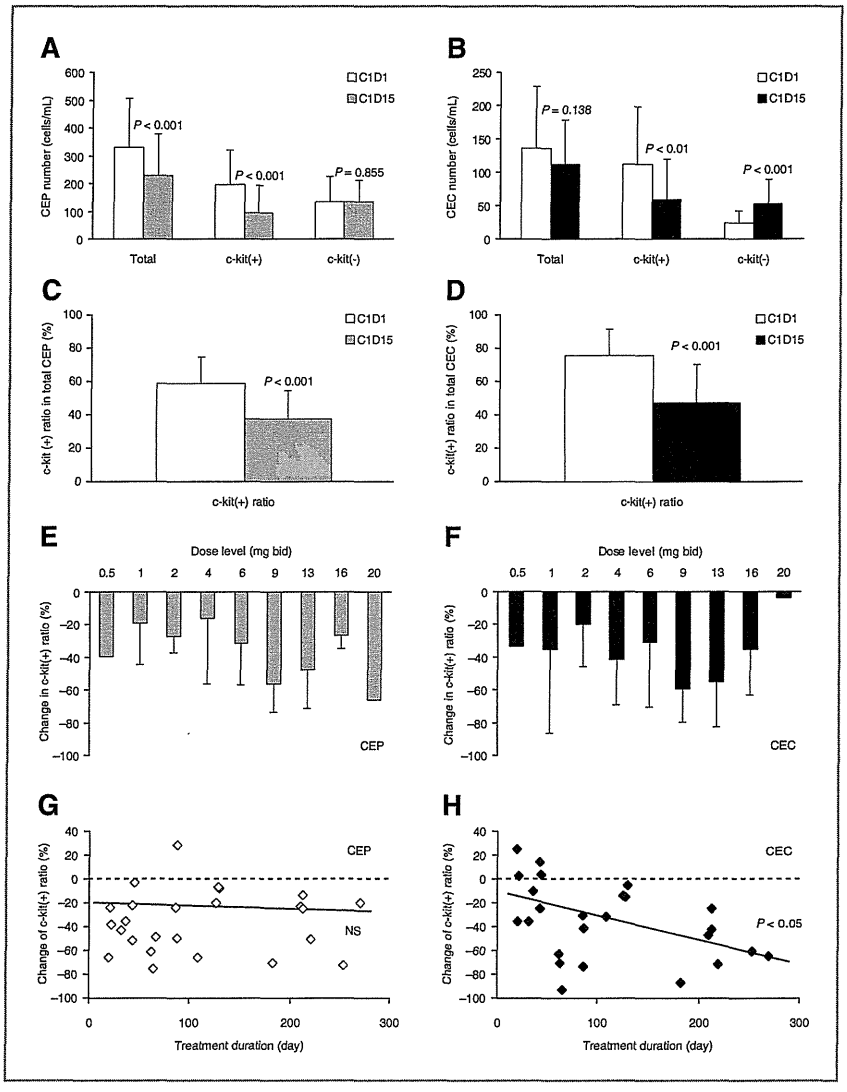

Figure 2. Decrease of CEP and CEC number associated with E7080 and correlation with treatment duration. A, 14-day E7080 treatment decreased total CEP, c-kit CEP, but not c-kit(−) CEP. B, E7080 treatment did not affect total CEC number, but decreased c-kit(+) CECs, and increased c-kit(−) CECs. C and D, 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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Phase II Study of Bolus 5-Fluorouracil and Leucovorin Combined with Weekly Paclitaxel as First-Line Therapy for Advanced Gastric Cancer

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Key Words
Gastric cancer · Chemotherapy · Weekly paclitaxel · Bolus 5-fluorouracil · Leucovorin

Abstract
Objective: We evaluated the efficacy and safety of bolus 5-fluorouracil (5-FU) and leucovorin combined with weekly paclitaxel (FLTAX) in advanced gastric cancer (GC) patients. ***Methods:*** Patients with untreated stage IV GC received paclitaxel 80 mg/m^2 as a 1-hour infusion, followed by 5-FU 600 mg/m^2 as a bolus infusion and L-leucovorin 250 mg/m^2 as a 2-hour infusion on days 1, 8 and 15. Treatment cycles were repeated every 28 days. The primary endpoint was response rate. ***Results:*** Thirty-five patients were enrolled. The median age was 62 years (range 34–75). Twenty-one patients (60%) had diffuse-type cancer and 11 had peritoneal metastasis. The confirmed response rate was 43% (95% CI 26–61) with 15 partial responses. Stable disease was observed in 16 (46%) patients. Median progression-free survival and overall survival were 6.8 months (95% CI 5.8–7.4) and 16.2 months (95% CI 10.0–22.8), respectively. Grade 3–4 adverse events were: neutropenia (54%), febrile neutropenia (3%), diarrhea (6%) and sensory neuropathy (11%). ***Conclusion:*** FLTAX showed a desirable safety profile, and the efficacy against advanced GC was encouraging. FLTAX may be a good option for GC patients with deteriorated general condition, and a randomized clinical trial in such patients is currently underway.

Introduction

Gastric cancer (GC) is the second leading cause of cancer death both worldwide and in Japan [1, 2]. Even though the incidence of GC is declining, approximately 930,000 cases are newly diagnosed each year worldwide [1]. Because of the vague and nonspecific symptoms associated with GC, the disease is often advanced on diagnosis, after which chemotherapy is the main treatment option. Oral fluoropyrimidines plus cisplatin-containing therapy is now considered to be the standard for advanced GC patients in most countries [3–5]. However, GC patients who are in poor condition or who have peritoneal dissemination cannot tolerate aggressive hydration and severe toxicity. Therefore, treatment with cisplatin is not indicated

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in a considerable portion of GC patients. Furthermore, patients with advanced GC often have gastrointestinal symptoms, negatively affecting their oral intake and sometimes precluding the administration of oral drugs. Intravenous administration-based therapies with mild toxicity and sufficient efficacy are needed.

Despite optimal treatment, advanced GC remains an aggressive malignancy, with a median survival of 9–13 months [3, 6, 7]. Recently, trastuzumab was shown to be the first molecularly targeted agent to provide survival benefits in HER2-positive GC patients [8]. However, HER2, the target of trastuzumab, is reportedly overexpressed only in approximately 20% of GC tumors, and the majority of these are of intestinal type [9, 10], which means patients with diffuse-type GC are not treated with trastuzumab. Previous reports have indicated that patients with diffuse-type GC have a significantly shorter survival than those with intestinal-type tumors, mainly due to the higher malignancy grade, with a high incidence of peritoneal metastases and deteriorated general condition [9, 11, 12]. Therefore, new chemotherapy regimens to improve the outcome in GC patients, particularly in diffuse-type cancer, are required.

Paclitaxel is pharmacologically and clinically considered to be effective against diffuse-type GC, as well as for intestinal types [13–15]. Because a weekly regimen of paclitaxel is less toxic than paclitaxel given once every 3 weeks [16], weekly regimens have become common in Japan, producing good results in GC patients [17]. 5-Fluorouracil (5-FU) is generally accepted as a key drug in the treatment of GC patients. Based on a sequence-dependent, synergistic cytotoxic effect of paclitaxel followed by 5-FU without overlapping toxicity [18, 19], we developed a chemotherapy regimen consisting of leucovorin-modulated weekly bolus 5-FU combined with weekly paclitaxel (FLTAX) on an outpatient basis. We subsequently conducted a phase I study to determine the recommended dose for the phase II study [20]. Preliminary safety data for the FLTAX regimen showed only mild toxicity.

This multi-institutional phase II study was designed to evaluate the efficacy and safety of the new non-platinum regimen of FLTAX as a first-line treatment for patients with metastatic or recurrent GC. The primary objective was to determine the overall response rate.

Patients and Methods

Patient Eligibility

This was a prospective, multi-institutional phase II clinical trial performed at the National Cancer Center Hospital, Kochi Health Sciences Center, Kanagawa Cancer Center, and Mitsubishi Kyoto Hospital. To be eligible, patients had to meet the following criteria: histologically proven unresectable or recurrent GC; age of 20–75 years; performance status of ≤2 according to the Eastern Cooperative Oncology Group scale; estimated life expectancy of >8 weeks after study entry, no prior chemotherapy for metastatic disease; adequate hematological function (white blood cell count between 3,000 and 12,000/mm^3, platelet count of ≥100,000/mm^3); adequate hepatic function (serum total bilirubin level of ≤2.0 mg/dl, AST and ALT levels of ≤100 IU/l); adequate renal function (serum creatinine level of ≤1.5 mg/dl); serum C-reactive protein level of ≤10 mg/dl; and written informed consent. Patients also had to have radiographically measurable disease according to the Response Evaluation Criteria in Solid Tumors guidelines [21]. Adjuvant chemotherapy with an oral fluoropyrimidine alone, not exceeding 1-year duration, completed more than 6 months before entry, was allowed.

Exclusion criteria were watery diarrhea; marked pleural effusion or ascites; active infection; severe comorbidity such as heart disease or renal disease; metastasis to the central nervous system; mental disorder; history of alcoholic hypersensitivity; active concomitant malignancy; pregnant or nursing women, and women of childbearing age, unless they were practicing effective contraception. This study was approved by the Institutional Review Boards of all participating institutes.

Treatment Plan

Paclitaxel (Taxol; Bristol-Myers K.K., Tokyo, Japan) at a dose of 80 mg/m^2 was administered as a 1-hour intravenous infusion followed by 5-FU (5-FU; Kyowa Hakko Kogyo Co., Ltd., Tokyo, Japan) 600 mg/m^2 as a bolus intravenous infusion on days 1, 8 and 15 of a 28-day cycle. A 2-hour intravenous infusion of L-leucovorin (Isovorin; Wyeth K.K., Tokyo, Japan) 250 mg/m^2 in normal saline solution was started at the same time as the paclitaxel infusion on the same days. This treatment was repeated until disease progression or unacceptable toxicity occurred. Short-term premedication was given to prevent paclitaxel-associated hypersensitivity reactions 30 min before the infusion of paclitaxel: dexamethasone 8 mg, ranitidine 50 mg and chlorpheniramine 10 mg.

Dose Attenuation

If patients had leukocytes <2,500/mm^3, platelets <100,000/mm^3, total bilirubin >2.0 mg/dl, AST and ALT >100 IU/l, or serum creatinine >1.5 mg/dl, both 5-FU/leucovorin and paclitaxel were withheld until recovery. To receive a subsequent cycle of chemotherapy, patients had to have a leukocyte count of ≥3,000/mm^3 and the recovery of any treatment-related nonhematological toxicity to grade ≤1 (except alopecia and neuropathy). Treatment was delayed for no more than 3 weeks to allow patients to recover from toxicities.

If a patient developed one of the following toxicities, the dose of 5-FU was reduced to 500 mg/m^2 for the subsequent cycle of treatment: grade 4 neutropenia lasting for 4 or more days; grade 3–4 thrombocytopenia; grade 3–4 febrile neutropenia; grade 3–4 diarrhea despite adequate antidiarrheal medication; any grade 3–4 nonhematological toxicity (excluding anorexia, nausea, vomiting, electrolyte abnormalities, and alopecia); treatment interruption for ≥2 weeks, and a delay of the start of the second cycle by ≥8 days because of toxicity. Any patient who required more

dose reduction of the FLTAX regimen was withdrawn from the treatment protocol.

Toxicity and Response Evaluation

Treatment-related toxic effects were assessed according to the National Cancer Institute Common Terminology Criteria for Adverse Events, version 3.0. During treatment, patient histories were obtained, and physical examination, complete blood counts with differential counts, serum chemical analyses, and urinalyses were carried out at least once a week. Tumor response was evaluated according to Response Evaluation Criteria in Solid Tumors guidelines [21] every 8 weeks until tumor progression.

Statistical Analysis

The primary endpoint of this study was overall response rate. In a design with an unacceptable response rate of 20%, an acceptable response rate of 40%, a statistical power of 80% and an α-error of 0.05, 35 patients needed to be enrolled and treated. Our secondary endpoints were progression-free survival time (PFS), time to treatment failure (TTF), overall survival time (OS), and toxicity. PFS was defined as the time from the date of starting treatment to the date of first documentation of disease progression or death. PFS in patients with protocol treatment cessation for toxicity was calculated as the time to the date of first documentation of disease progression in subsequent therapies. TTF was measured from the date of starting treatment to the date of treatment cessation for any reason. OS represented the duration from the date of starting treatment to the date of death from any cause. PFS, TTF and OS were calculated by the Kaplan-Meier method. All statistical analyses were performed using JMP software (version 9.0.1; SAS Institute Inc., Cary, N.C., USA), with the final analysis conducted in January 2011. This study was registered with UMIN-CTR (ID number: UMIN000000502).

Results

Patient Characteristics and Drug Delivery

Thirty-five patients were enrolled in this study between September 2006 and October 2009. Response and toxicity were assessable in all patients. The clinical characteristics of the patients are shown in table 1. The majority of patients were males (86%), with a median age of 62 years (range 34–75). More than half of patients had diffuse-type GC (60%), and 77% had unresected primary tumor.

The median number of treatment cycles per patient was 5 (range 1–9; table 2). At the time of analysis, all patients had completed protocol treatment. The median relative dose intensity during the protocol treatment was 82% (range 55–100) for 5-FU, 88% (range 58–100) for L-leucovorin and 88% (range 60–100) for paclitaxel. After a 1-week observation period in the hospital, all patients were able to receive treatment on an outpatient basis.

Table 1. Patient characteristics

	Patients	
	n	%
Total patients	35	100
Sex		
Male	30	86
Female	5	14
Age, years		
Median	62	
Range	34–75	
ECOG performance status		
0	16	46
1	19	54
2	0	0
Histological type		
Intestinal	14	40
Diffuse	21	60
Prior surgery		
None	27	77
Gastrectomy	8	23
Adjuvant chemotherapy	0	0
Site of metastasis		
Lymph nodes	35	100
Liver	21	60
Peritoneum	11	31
Lung	4	11
Adrenal gland	1	3
Subsequent lines of chemotherapy		
2nd line	32	91
3rd line	14	40
4th line and more	6	17
Unknown	2	6

ECOG = Eastern Cooperative Oncology Group.

Efficacy

All 35 patients were included in the evaluation of response. Confirmed tumor response was partial response in 15, stable disease in 16, and progressive disease in 4 subjects (table 2). The confirmed overall response rate was 43% [95% confidence interval (CI) 26–61], and the best overall response rate including the unconfirmed partial response was 63% (95% CI 45–79). Four patients (11%) underwent secondary curative resection after major response to protocol treatment, and 2 patients underwent surgery before confirmation of partial response. No patients had a complete response.

At a median follow-up time of 17.8 months, 23 patients (66%) had died. The median PFS and TTF were 6.8 months (95% CI 5.8–7.4) and 5.6 months (95% CI 3.1–6.2), respectively (fig. 1a). The median OS was 16.2

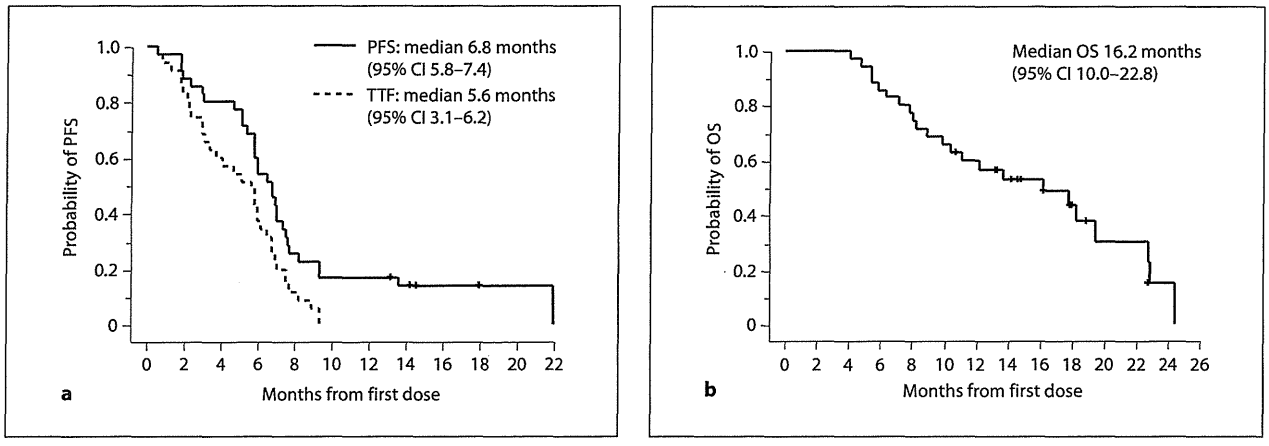

Fig. 1. Kaplan-Meier curves for PFS and TTF (**a**) and OS (**b**) in all patients (n = 35).

months (95% CI 10.0–22.8). Thirty-two (91%) patients received second-line chemotherapy (table 1). Among these, 15 patients were given cisplatin-based therapy, 8 patients S-1 alone, 4 patients 5-FU plus leucovorin, 3 patients irinotecan alone, and 2 patients paclitaxel-based therapy.

In an exploratory subgroup analysis, the best response rate and PFS in patients with diffuse-type GC were 62% (95% CI 38–82) and 6.8 months (95% CI 5.1–8.2), respectively. On the other hand, these results in patients with intestinal-type cancer were 64% (95% CI 35–87) and 7.0 months (95% CI 1.9–7.7), respectively. The median OS was 17.8 months (95% CI 8.2–19.5) in diffuse-type GC and 16.2 months (95% CI 7.2 to infinite) in intestinal-type GC. There were no significant differences in response rate, PFS or OS among the histological types.

Toxicity

Major toxic effects occurring during the protocol treatment are summarized in table 3. The most common grade 3–4 toxicity was neutropenia (54%). One patient developed neutropenic fever and recovered with appropriate therapy. No patients developed grade 3–4 thrombocytopenia. Nonhematologic toxicities observed in the present study were generally mild. Only a small portion of patients experienced grade 3 nonhematologic toxicities: diarrhea in 2 patients (6%) and sensory neuropathy in 4 patients (11%; table 3). Although grade ≥1 diarrhea developed in 21 patients (60%), it was mild and promptly resolved after appropriate medical treatment such as with antidiarrheal agents. There was no grade 4 nonhematologic toxicity or treatment-related death in the present study. As for cumulative toxicity, 12 patients (34%) had grade ≥2 sensory neuropathy. In 6 of these patients, the treatment protocol was discontinued because of prolonged sensory neuropathy, and the median number of cycles delivered before discontinuation was 4.5 (range 2–7). All grade ≥2 neuropathy patients recovered within 2 months after treatment discontinuation. No other cumulative toxicity was observed.

Table 2. Drug delivery and treatment response

	Median	Range
Drug delivery		
Chemotherapy cycles	5	1–9
Relative dose intensity		
5-FU	0.82	0.55–1.00
L-Leucovorin	0.88	0.58–1.00
Paclitaxel	0.88	0.60–1.00
Confirmed tumor response[1]		
Total	35 (100)	
CR	0	
PR	15 (42.9)	
SD	16 (45.7)	
PD	4 (11.4)	
Overall response rate, %	42.9	
Overall response rate, 95% CI	26.3–60.7	
Best overall response rate, %	62.9	
Best overall response rate, 95% CI	44.9–78.5	

Figures in parentheses are percentages. CR = Complete response; PR = partial response; SD = stable disease; PD = progressive disease.

[1] According to Response Evaluation Criteria in Solid Tumors.

The reasons for cessation of protocol treatment were progressive disease in 23 patients (66%), sensory neuropathy in 6 (17%), radical gastrectomy after tumor regression in 4 (11%), and infection in 2 (6%). One patient with a history of mild emphysema discontinued the protocol treatment and switched to another chemotherapy regimen because of recurrent pneumonia. In another patient, the protocol treatment had to be withdrawn at the end of the second cycle because of grade 3 diarrhea and infectious colitis.

Table 3. Number of patients with toxicity

Toxicity	By patient (n = 35)		By cycle (n = 154)	
	all grades	grade 3/4	all grades	grade 3/4
Hematologic toxicity				
Leukopenia	32 (91)	9 (26)	114 (74)	12 (8)
Neutropenia	31 (89)	19 (54)	103 (67)	38 (25)
Anemia	35 (100)	7 (20)	150 (97)	15 (10)
Thrombocytopenia	6 (17)	0	9 (6)	0
Febrile neutropenia	–	1 (3)	–	1 (1)
Nonhematologic toxicity				
Nausea	23 (66)	0	57 (37)	0
Vomiting	9 (26)	0	18 (12)	0
Diarrhea	21 (60)	2 (6)	49 (32)	2 (1)
Stomatitis	12 (34)	0	19 (12)	0
Skin rash	7 (20)	0	12 (8)	0
Sensory neuropathy	30 (86)	4 (11)	94 (61)	4 (3)
Hand-foot syndrome	11 (31)	0	32 (21)	0
Alopecia	31 (89)	–	112 (73)	–
AST elevation	19 (54)	0	34 (22)	0
ALT elevation	13 (37)	0	24 (16)	0
ALP elevation	17 (49)	0	34 (22)	0
T-bil elevation	0	0	0	0

Figures in parentheses are percentages. T-bil = Total bilirubin.

Discussion

Most patients with advanced GC receive chemotherapy as a part of palliative therapy. Although oral fluoropyrimidine plus cisplatin-containing regimens are considered to be the standard for advanced GC, such regimens are toxic and cannot be tolerated by many patients with advanced GC because of poor performance status at initial diagnosis. Therefore, a chemotherapy regimen with the least toxicity and sufficient efficacy is desirable, and treatment on an outpatient basis is ideal for patient quality of life. Before conducting clinical trials of FLTAX in patients with deteriorated general condition, we needed to confirm its antitumor activity in advanced GC patients with relatively good performance status [20]. Our current multi-institutional phase II study of the FLTAX regimen showed that the best response rate was 63% and median PFS and OS were 6.8 and 16.2 months, respectively. The toxicity profile of FLTAX was similar to those in previous studies using different administration schedules for protracted 5-FU infusion and paclitaxel [22–25]. The incidence of grade 3 sensory neuropathy (11%) in our study was also identical to previous reports on weekly paclitaxel [26, 27]. Accordingly, FLTAX for advanced GC patients would be a good option and has the advantage of avoiding the use of indwelling venous access devices or ambulatory pumps for outpatient treatment. Furthermore, as the toxicity profile of FLTAX was found to be favorably mild, the FLTAX regimen is also a promising substitute therapy for GC patients who cannot tolerate cisplatin-containing therapy.

Oral fluoropyrimidines such as S-1 and capecitabine have been evaluated in numerous large-scale clinical trials [4–6, 28]. However, they cannot be used in a considerable portion of patients with advanced GC. For example, tumors associated with obstruction of the pylorus or cardia or with peritoneal dissemination as a common characteristic of diffuse-type GC cause dysphagia, nausea, vomiting and intestinal obstruction, often precluding the administration of oral anticancer drugs. Recently, the non-inferiority of leucovorin-modulated weekly bolus 5-FU regimen in comparison with S-1 [29] and good anti-tumor activity of S-1 plus docetaxel [30] in advanced GC patients have been reported in phase III trials. Unfortunately, there are no data directly comparing the antitumor activity of 5-FU plus taxane (paclitaxel or docetaxel) with that of S-1 plus taxane. Although there are limitations in comparing the results of different studies, the efficacy of S-1 plus paclitaxel has been reported in phase II trials with a response rate of 40–55% and a median survival of 9–15 months, and this is comparable to the present data for FLTAX [31–33]. Accordingly, the FLTAX regimen may be a good option for GC patients who are not suitable for treatment with platinum agents.

The median OS in our study was slightly longer than those in previous phase II/III studies for 5-FU or S-1 combined with taxane, although the median PFS was comparable [25, 30–32]. The longer OS in the present study may be related to the better performance status of patients enrolled as compared with that in other studies. Furthermore, most patients in our study received subsequent lines of chemotherapy (table 1). As second-line chemotherapy, 15 patients were given cisplatin-based therapy (cisplatin plus irinotecan for 10, cisplatin plus S-1 for

4, and cisplatin plus 5-FU for 1 patient). Both better performance status and the additional lines of chemotherapy administered in the present study may have contributed to the better OS outcome, as reported previously [34, 35].

The survival outcome of patients with diffuse-type GC is reportedly worse than that of subjects with the intestinal type [9, 36]. Diffuse-type GC has higher malignancy grades, is more resistant to chemotherapy, is more likely to metastasize to the peritoneum causing intestinal obstruction and ascites, and consequently, is more likely to exhibit a poor performance status [11, 12, 37–39]. Therefore, a new effective therapy for diffuse-type GC patients with deteriorated condition is needed. In the subgroup analysis of the present study, the response rate, PFS and OS of FLTAX in diffuse-type GC (21 patients) were favorable and similar to those in intestinal-type cancer (14 patients). The FLTAX regimen would be an effective regimen for GC, irrespective of histological type. The favorable toxicity profile of FLTAX may make it a viable alternative treatment for patients who cannot receive intensive standard platinum regimens. The good toxicity profile might also permit the concurrent use of new targeted agents in such patients.

Subsequent phases of the present study for FLTAX are currently underway. First, a multi-institutional feasibility study of FLTAX for GC patients with massive ascites or inadequate oral intake was conducted [40]. Based on the dose-finding part of the feasibility study (n = 13), the recommended dose was set at 5-FU/L-leucovorin/paclitaxel = 500/250/60 mg/m^2. A subsequent part of the study (n = 25) showed that the objective response rate of ascites was 44%, the ascites control rate was 96%, and the toxicity profile of FLTAX was acceptable in these patients [40]. A randomized clinical trial is currently being planned to examine whether the FLTAX regimen can be a standard first-line treatment for GC patients with massive ascites or inadequate oral intake.

In conclusion, the present multi-institutional phase II study demonstrated that FLTAX would be an active chemotherapy regimen with satisfactory efficacy and safety in advanced GC patients. The desirable toxicity profile of this encouraging non-platinum regimen will serve as the basis for establishing FLTAX as a standard therapy for GC patients in deteriorated general condition, and further clinical trials are warranted.

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Disclosure Statement

We declare no conflicts of interest.

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