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, Cmax 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,
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.

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 ≤100 mmHg.
hypertension not controlled by any antihypertensive drugs
more nonhematologic toxicity (with exceptions of grade 4 platelet count decrease, grade 4 neutropenia, any grade 3 or
(ment was eligible for treatment at a reduced dose level
DLT that resolved sufficiently to allow continued treat-
delayed for 14 or less days. Any patient who experienced a
recover from any toxicities, a treatment cycle could be
second dose reductions, respectively). A maximum of up
to 2 dose reductions was permitted.

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 treat-
ment 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 antiarrheal drugs), and failure to admin-
ister 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 biochem-
istry, and blood coagulation), urinalysis, electrocardio-
gram, 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 spectro-
metry (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 angiogen-
esis (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 isothio-
cyanate (FITC)-conjugated anti-human CD34, FITC-con-
jugated anti-human CD45, phycoerythin-conjugated
anti-human CD117 (c-kit), and with FITC-conjugated
anti-human CD133. Cells were then washed with phos-
phate-buffered saline and fixed in 4% paraformaldehyde,
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 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)

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

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. 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
(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 ($C_{\text{max}}$), the time to peak plasma concentration ($t_{\text{max}}$) and elimination half-life ($t_{1/2}$) for E7080 after a single dose and during steady state (ss) were similar (Table 3). $C_{\text{max}}$ and area under the plasma concentration–time curve (AUC) from time zero to the last measurable concentration (AUC0–t) were dose proportional (Fig. 1).

The serum protein binding rates ranged from 96.6% to 98.2%. The previously reported IC50 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 IC50 of E7080 in plasma was almost equivalent to a maximum plasma concentration ($C_{\text{max}}$) at 0.5 mg bid and to a minimum plasma concentration ($C_{\text{min}}$) at 2 mg bid in multiple dosing (Table 2). After

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

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

Abbreviations: GGT, γ-glutamyltransferase; TSH, thyroid stimulating hormone; WBC, white blood cells.
### 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 of a 21-day cycle (cycle 1)

<table>
<thead>
<tr>
<th>Parameterb</th>
<th>E7080 dose levels, mg bid</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.5</td>
</tr>
<tr>
<td>Cycle 0 (single dose)d</td>
<td>n</td>
</tr>
<tr>
<td>Cmax, ng/mL</td>
<td>2.5 (0.2)</td>
</tr>
<tr>
<td>tmax, h</td>
<td>5 (3–9)</td>
</tr>
<tr>
<td>AUC0–24, ng h/mL</td>
<td>41 (2.0)</td>
</tr>
<tr>
<td>AUC0–24, ng h/mL</td>
<td>115 (2.7)</td>
</tr>
<tr>
<td>t1/2, h</td>
<td>46.5 (5.9)</td>
</tr>
<tr>
<td>Cycle 1 (multiple dosing)e</td>
<td>n</td>
</tr>
<tr>
<td>Cmax, ng/mL</td>
<td>16.7 (5.2)</td>
</tr>
<tr>
<td>Cmin, ng/mL</td>
<td>7.2 (2.6)</td>
</tr>
<tr>
<td>tmaxss, h</td>
<td>1 (1–3)</td>
</tr>
<tr>
<td>AUC0–tss, ng h/mL</td>
<td>128 (36)</td>
</tr>
<tr>
<td>t1/2ss, h</td>
<td>37.1 (1.0)</td>
</tr>
</tbody>
</table>

| E7080 administered only in the morning of day 14, cycle 1. |
| Data are shown as mean (SD), except for tmax and tmaxss which are median (range). |
| For E7080 20 mg bid, individual values are displayed for each patient. |
| The pharmacokinetic profile was evaluated in cycle 0 after single dosing in 26 patients. |
| The pharmacokinetic profile was evaluated in cycle 1 after multiple dosing in 25 patients. |

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*aE7080 administered only in the morning of day 14, cycle 1. 
*bData are shown as mean (SD), except for *t*max and *t*maxss which are median (range).
*cFor E7080 20 mg bid, individual values are displayed for each patient.
*dThe pharmacokinetic profile was evaluated in cycle 0 after single dosing in 26 patients.
*eThe pharmacokinetic profile was evaluated in cycle 1 after multiple dosing in 25 patients.
repeated E7080 administration, urinary excretion of the parent compound (feo–) 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 \( (P = 0.27) \). 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 \( (P < 0.01) \), although this was independent of E7080 dose \( (P = 0.18) \). The reduction in c-kit(+) ratio in CECs associated with E7080 treatment correlated with treatment duration \( (\text{Spearman’s rank correlation coefficient } \rho = -0.468; P = 0.018) \), while no correlation of c-kit(+) ratio in CEPs with treatment duration was observed \( (P = 0.20) \).

*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 \( (P < 0.05) \) (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 \( (P < 0.01) \) and predose levels of plasma SDF1α \( (P < 0.05) \) (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
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.

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.
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 DLIs 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, NCT00-946153, 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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