Results of a Phase I Dose-escalating Study of the Antiangiogenic Agent, SU5416, in Patients with Advanced Malignancies

Alison Stopeck,[1] Marrae Sheldon, Mahmood Vahedian, Gillian Cropp, Rishi Gosalia, and Alison Hannah

Arizona Cancer Center, Tucson, Arizona 85724-5024 [A. S., M. S., M. V., R. G.], and SUGEN, Inc., South San Francisco, California 94080 [G. C., A. H.]

ABSTRACT

SU5416 is a small molecule antiangiogenic agent that inhibits vascular endothelial growth factor (VEGF) stimulation of the KDR tyrosine kinase receptor. In this Phase I dose escalation trial, a weekly dose schedule of SU5416 was tested whereby an initial 5-day loading dose was followed by weekly maintenance infusions. The start dose was 20 mg/m^2 for the loading dose followed by 65 mg/m^2 for the weekly infusions. Dose escalations occurred at 33% until a final dose of 65 mg/m^2 (loading dose) and 190 mg/m^2 (weekly infusion) was obtained. Twenty-two patients were treated at five dose levels; tumor types included gastrointestinal (8), breast (3), lung (4), sarcoma (2), and other (5). The most common serious drug-related toxicity was headache, often associated with nausea and vomiting. Grade 1 and 2 toxicities included headache, nausea, vomiting, asthenia, pain at the infusion site, phlebitis, change in voice, and fevers. Of 19 evaluable patients, 4 obtained clinical benefit as defined by tumor regression (1) or disease stabilization for at least 12 weeks (3). Pharmacokinetic data revealed that the weekly infusion schedule prevented the reported 50–60% induction in SU5416 clearance observed with either daily or twice weekly dosing. Higher baseline levels of urine VEGF were observed in the 4 patients who gained clinical benefit, suggesting this may be a useful marker for predicting response to anti-VEGF therapies. Our results suggest that a weekly schedule of SU5416 shows signs of biological activity and is well tolerated at doses up to 145 mg/m^2.

INTRODUCTION

Tumor-induced angiogenesis is a multistep process by which endothelial cells form the new network of vessels required for tumor growth and metastatic spread. Increased angiogenesis in tumors has been associated with poor prognosis in numerous tumor types (1–5). In addition, tumors have been found to secrete multiple inducers of angiogenesis (reviewed in Refs. 6–8). The expression of inducers and inhibitors of angiogenesis is often regulated by oncogenes and tumor suppressor genes, including *p53*, suggesting a link between angiogenesis and tumorigenesis (9, 10).

Paracrine stimulators derived from tumor cells are the main inducers of endothelial cell migration, proliferation, and new vessel formation. Of the many known inducers, VEGF[2] is thought to play the most important role, resulting in endothelial cell proliferation and migration (11). VEGF is also believed to serve as a survival factor required for the maintenance of new vessels (12). Increased VEGF expression and secretion has been found in most tumor subtypes (13, 14).

VEGF actions on endothelial cells are primarily mediated via its two tyrosine kinase receptors, VEGF receptor 1 (VEGFR-1/Flt-1) and VEGF receptor 2 (VEGFR-2 and Flk-1/KDR), which are localized on the surfaces of endothelial cells (11). In animal models, investigators have shown that inhibition of the VEGF pathway can induce tumor regression and prevent metastases (15–19). There are multiple approaches to inhibiting VEGF activity including antisense or ribozymes that target either VEGF or VEGF receptor mRNA, small molecules that inhibit tyrosine kinase receptors, including specifically the VEGF receptors, soluble recombinant VEGF receptors that bind to circulating VEGF and prevent VEGF from binding to endothelial receptors, and antibodies that directly neutralize VEGF or block its receptors (16, 19–23)

SU5416 (semaxanib) is a small molecule inhibitor of the VEGF receptor 2 (Flk-1/KDR) tyrosine kinase (Fig. 1). SU5416 has been shown to inhibit VEGF-dependent endothelial cell proliferation *in vitro* and in animal models (24). Phase I and II clinical trials of SU5416 have been completed using a twice-weekly dosing regimen, with a MTD equal to 145 mg/m^2. The DLT consisted of severe headaches with nausea and vomiting, lasting 1–2 days, and refractory to analgesics, antiemetics, and antimigraine therapies (25, 26). In murine models, administration of SU5416 by either a twice-weekly or weekly dosing after a 5-day load produced similar inhibition of s.c. tumor growth (24). Later preclinical studies demonstrated efficacy in certain tumor lines with only weekly dosing (27). Because a weekly dosing schedule has significant quality of life advantages, we initiated a Phase I dose-escalation trial using a 5-day load followed by five weekly maintenance infusions to determine the MTD using this novel dosing regimen. We also obtained additional information on the antitumor activity of SU5416 in multiple solid tumor types, measured urinary VEGF and bFGF

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[1] To whom requests for reprints should be addressed, at Arizona Cancer Center, P.O. Box 245024, Tucson, AZ 85724-5024. Phone: (520) 626-2816; Fax: (520) 626-3754.

[2] The abbreviations used are: VEGF, vascular endothelial growth factor; DLT, dose-limiting toxicity; MTD, maximum tolerated dose; bFGF, basic fibroblast growth factor; CT, computed tomography; AUC, areas under the concentration time curve.

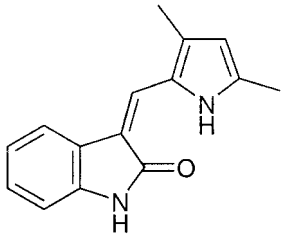

Fig. 1 Chemical structure of SU5416.

Table 1 Dose escalation scheme of SU5416

Dose level	Loading dose days 1–5	Weekly maintenance dose	No. patients treated	No. 6-week cycles completed
1	20 mg/m^2	65 mg/m^2	4	4
2	27 mg/m^2	85 mg/m^2	4[a]	8
3	36 mg/m^2	113 mg/m^2	6	7
4	48 mg/m^2	145 mg/m^2	6	10
5	65 mg/m^2	190 mg/m^2	3	4
	Total		22	33

[a] Patient 003 received two cycles at dose level 1 and one cycle at dose level 2.

levels as surrogate markers of biological activity, and determined the pharmacokinetic and toxicity profile of SU5416 in a weekly dosing regimen.

PATIENTS AND METHODS

Patient Selection. Patients with histologically documented malignancies refractory to standard therapy or for whom no effective therapy existed were eligible for this study. Twenty-two patients were enrolled at the Arizona Cancer Center from September 14, 1998 through October 25, 1999. Relevant eligibility criteria included: age >18 years; Karnofsky performance status of at least 60%; no chemotherapy, radiation therapy, or investigational agent within 4 weeks (6 weeks for nitrosoureas or mitomycin); adequate hematopoietic: ANC ≥1,500/μl, hemoglobin ≥9 mg/dl, platelet count >100,000/μl; hepatic: total bilirubin ≤1.5 × the upper limit of normal; aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase ≤3 × upper limit of normal, and renal function: serum creatinine <2.0 mg/dl. A prestudy D-dimer was also obtained in 20 of the 22 patients. Informed consent was obtained before enrollment on study according to federal and institutional guidelines.

Drug Administration. The starting dose of SU5416 was 20 mg/m^2/day during the 5-day loading period followed by weekly infusions at 65 mg/m^2. Use of a lower dose during the 5-day load was based on the results from previous animal studies (24). Dose escalations for both the loading dose and weekly doses occurred at 33% until a final dose of 65 mg/m^2/day (loading dose) and 190 mg/m^2 (weekly infusion) was obtained (see Table 1). Dose escalations continued until DLT was noted or until the maximum dose of 190 mg/m^2 weekly infusion was reached (a dose of 145 mg/m^2 results in similar systemic exposure to that observed in the tumor xenograft model and similar free plasma concentration to that used in the *in vitro* endothelial cell proliferation assay). The MTD was defined as one dose level below the dose that induced DLT in >1 of 3 new patients. DLT was defined as grade III-IV nonhematologic toxicity or grade IV hematologic toxicity. Toxicities were graded according to the National Cancer Institute common toxicity criteria. Intraindividual dose escalations were permitted in subjects who experienced minimal toxicity, provided that 3 new patients had completed a minimum of two weekly infusions of SU5416 at the next higher dose without DLT.

Patients with central venous access catheters or positive prestudy D-dimer assays were encouraged to receive oral coumadin 1 mg/day unless contraindicated. Patients with other significant risk factors for venous thrombosis including history of venous thrombosis, obesity, marked varicose veins, or protracted immobility were also offered treatment with up to 2.5 mg/day of coumadin.

SU5416 was supplied by SUGEN, Inc. (South San Francisco, CA) as a yellow-orange sterile parenteral formulation in 30-ml vials containing 112.5 mg of SU5416 in 25 ml of solution (4.5 mg/ml final concentration). Additional components of the formulation included: polyethylene glycol 400, polyoxyl 35 castor oil (Cremophor), benzyl alcohol, and dehydrated alcohol. The drug was diluted 1:3 with 0.45% sodium chloride before administration. Because the drug contains Cremophor, it was infused using non-PVC-lined i.v. bags and administration sets at a rate of 200 cc/h. Before infusion, all of the patients received premedication including diphenhydramine 50 mg or loratadine 10 mg, cimetidine 300 mg, and dexamethasone 10–20 mg administered i.v. 1 h before study drug. The dose of dexamethasone was decreased to 4 mg as tolerated.

Plasma Sampling. Blood for the determination of parent drug, SU5416, was collected after the first, fifth, and tenth infusion on days 1, 5, and 36 (after the first infusion, at the end of the 5-day loading dose, and after five weekly infusions of SU5416). Heparinized blood samples were drawn at the end-of-infusion, 5, 10, 20, 30, 45, 60, 120, and 240 min after the administration of SU5416 for all of the patients; patients enrolled in dose level #5 had additional blood samples drawn at 6, 8, 12, and 24 h after the infusion.

Plasma Analysis. The analytical laboratory that performs plasma SU5416 determinations is Specialty Laboratories (Santa Monica, CA). This laboratory operates under GLP and is certified by Clinical Laboratory Improvement Amendments, the State of California, and the College of American Pathologists.

The analytical method is high-performance liquid chromatography with a limit of quantitation of 10 ng/ml plasma. Briefly, 0.5-ml aliquots of heparinized plasma were added to the internal standard (5-chloro-SU5416), and the plasma was extracted with acetonitrile, centrifuged, and the supernatant decanted and evaporated to dryness under nitrogen. Residues are taken up into methanol/mobile phase and injected onto the chromatograph. High-performance liquid chromatography assay uses a linear gradient from 100% A to 100% B. Components of the mobile phase are: A, methanol:35 mM KH2PO4 buffer with 0.01% triethylamine (30:70); and B, 100% methanol. The gradient run time is 25 min; UV detection is set at 440 nm. The chromatography column is a Symmetry C18 (3.9 inside diameter × 100 mm) 5 μm particle size with Symmetry C18 guard column (3.9 × 20 mm) manufactured by Waters Corp. Under

Table 2 Patient characteristics

Characteristics	No.
No. of patients	22
No. evaluable	19
Sex (male/female)	15/7
Age, years	
Median	57
Range	29–78
Karnofsky performance status %	
90–100	15
70–80	7
Prior therapies	
Chemotherapy	19
Radiation alone	1
No prior therapy	2
Tumor type	
Gastrointestinal	8
Lung	4
Breast	3
Sarcoma	2
Other[a]	5

[a] Includes one each of the following: ovarian, melanoma, neuroendocrine, unknown primary, and myeloma.

Table 3 Grade 1 and 2 toxicities possibly/probably related to SU5416

Adverse event	No. of events (grade)		
	1	2	1 + 2
Headache	21	19	40
Vomiting	17	13	30
Nausea	21	5	26
Fatigue/weakness	15	8	23
Dehydration/light-headed	8	5	13
Fever	5	4	9
Burning at IV site	8	1	9
Diaphoresis	2	3	5
Hoarse voice	4	0	4
Diarrhea	3	1	4
Taste changes	2	1	3
Chest pain/tightness	3	0	3

these conditions retention times are ~18 min (SU5416) and 19 min (internal standard). The assay is linear from 10 to 2000 ng/ml with coefficients of determination 0.998–0.9999. Intra-assay coefficients of variation fall between 2 and 5.5% for SU5416 tested at low (50), medium (700), and high (1800 ng/ml) plasma concentrations. Interassay coefficient of variation is between 4 and 7% over the same range of concentrations. Accuracy is 94–100% for SU5416, and recovery of extracted compared with unextracted drug 97.1 ± 3.2% over the three concentrations.

Specificity of the methodology was determined by extracting blank human plasma ($n = 20$) and determining that no endogenous peaks coeluted at the retention times of interest. No interference was found with >30 therapeutic drugs (including the 3 medications used as premedication before SU5416 infusions) and 6 endogenous compounds (α- and β-carotene, vitamins A and K, and α- and β-tocopherol), which were tested in the analysis.

Trilevel quality control samples were developed to be included with each analytical run and monitored using Westgard rules, which interpret the control data and determine acceptance of each run. The assay was found to be linear, precise, accurate, and free of interference because of the biological matrix or potentially coadministered therapeutic drugs.

Pharmacokinetics. Modeling of the plasma concentration time data was performed using a nonlinear mixed effects program (NONMEM; NONMEM Project Group, University of California at San Francisco, San Francisco, CA), which uses an extended least squares algorithm. The model assumed a two-compartment disposition for SU5416 and allowed for changes in kinetic variables for each sampling period.

Quantification of Urinary VEGF and bFGF Levels. Twenty-four-h urine collections were obtained immediately pre-study, before the fifth daily dose of SU5416 during the loading week (cycle 1, day 5), and before receiving their week 6 (last dose of cycle 1) dose of SU5416. Aliquots of the urine were frozen at −70°C and batched for ELISA analysis. For a subset of 10 patients the first a.m. urine was analyzed separately before pooling for the complete 24-h urine collection. VEGF and bFGF levels (pg/ml) were measured using the Quantikine human VEGF (sensitivity <5 pg/ml) and Quantikine High Sensitivity human bFGF (sensitivity <0.25 pg/ml) ELISA kits (R&D Systems, Inc., Minneapolis, MN). The 24-h urinary secretion was then calculated by multiplying the pg/ml of VEGF or bFGF as determined by ELISA by the total quantity (in milliliters) of urine collected over 24 h.

RESULTS

Twenty-two patients were treated and received a total of 33 courses of therapy. All of the patients were evaluable for toxicity. Three patients were considered inevaluable for response as they completed <4 weeks of therapy. Patient characteristics are listed in Table 2. Patients received a median of one cycle of therapy with a range of 0–5 cycles completed.

Toxicities. There was no grade 4 toxicity observed. The major DLT was headaches frequently associated with nausea and vomiting that began several hours after receiving SU5416 and resolved completely within 48 h. Five patients developed grade 3 headaches associated with nausea and vomiting. The headaches generally were unresponsive to standard therapies including analgesics, antiemetics, and antimigraine therapies including sumatriptan succinate. Although headaches often recurred with subsequent administration of the drug, the severity decreased over time. Grade 1–2 headaches associated with nausea and vomiting were also common (see Table 3). Other significant toxicities included hypersensitivity reactions, most likely secondary to the Cremophor contained in the SU5416 drug product (see Table 4). One patient was discontinued from study after experiencing a severe anaphylactoid reaction including flushing, hypotension, and shortness of breath despite pretreatment with steroids and H_1 and H_2 blockers. The reaction resolved with i.v. hydration and repeat steroid administration. The only other grade 3 toxicity was acute renal failure in a patient receiving the 145 mg/m^2 dose. The patient was hospitalized with nausea and dehydration when his creatinine increased to 3.0 mg/dl. His creatinine returned to baseline after i.v. hydration and he was discharged in good condition after a 4-day hospitalization. Other reversible grade 1 and 2 toxicities in-

Table 4 Grade 3 toxicities possibly/probably related to SU5416

Adverse event	Patient no.	Dose level	Dose	No. events
Headache ± nausea/vomiting	5	2	85 mg/m^2	1
	8	3	113 mg/m^2	2
	9	3	113 mg/m^2	1
	19	4	145 mg/m^2	3
	22	5	190 mg/m^2	1
Hypersensitivity reaction	15	4	145 mg/m^2	1
Renal failure	14	4	145 mg/m^2	1

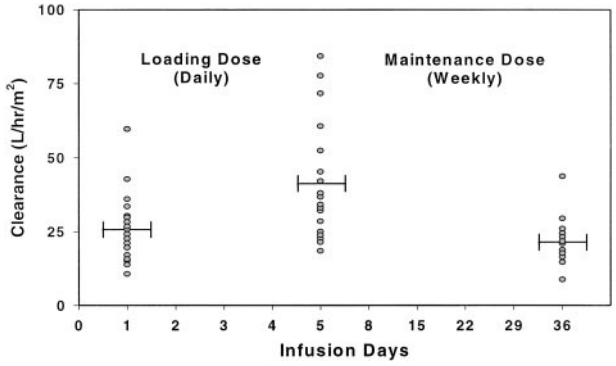

Fig. 2 Total systemic clearance (Clearance = liter/h/m^2) after the first and fifth daily loading dose infusions of SU5416, and after the fifth weekly infusion showing induction of clearance and return to baseline (mean indicated with *bar*).

cluded hoarse voice, diarrhea, taste changes, fever, and irritation or phlebitis of the infused peripheral vein when a central venous catheter was not available.

Patients received coumadin prophylaxis depending on their risk factors (as outlined in "Materials and Methods"). In all, 14 (64%) patients received coumadin 1 mg/day, and 1 patient with a prior history of deep venous thrombosis, obesity, severe varicose veins, and decreased mobility received coumadin 2.5 mg/day. No episodes of venous or arterial thrombosis were observed during the course of the study or follow-up period.

Antitumor Activity. Of the 22 patients treated, 19 were considered evaluable for antitumor responses. Four patients obtained clinical benefit as defined by tumor regression (1 patient) or disease stabilization for at least 12 weeks (3 patients). The best clinical responses were in 2 patients (patients #14 and #16) receiving the 145 mg/m^2 weekly dose. Patient #14 was a 70-year-old man diagnosed in 1991 with a right upper lobe mass. He initially underwent resection, which revealed squamous cell carcinoma. In February 1999, he recurred and was started on therapy with SU5416 at the 145 mg/m^2 weekly dose (dose level 4) in April 1999. His baseline right upper lobe mass measured 8 × 5.8 cm and decreased to 5.5 × 4.2 cm (50% decrease in the product of bidimensional measurements) on follow-up CT scan 8 weeks after commencing therapy consistent with a partial response. The patient was removed from study at week 8 per his request after a 4-day hospitalization for nausea, dehydration, and reversible acute renal failure. Two months after removal from study, he was started on salvage chemotherapy by his referring oncologist despite persistent stable disease on his chest CT scans.

Patient #16 is a 55-year-old man diagnosed in April 1997 with angiosarcoma involving the right iliac bone and femur. He was initially treated with radiation therapy to the involved area. On May 24, 1999, he was started on SU5416 (145 mg/m^2 weekly dose) for increasing pain and an enlarging mass as measured by magnetic resonance imaging. On therapy, the patient had marked improvement in his pain, a significant decrease in his pain medications, and disease stabilization by serial magnetic resonance imaging. After disease stabilization for >11 months (completing 5.5 6-week cycles), the patient was permanently withdrawn from protocol for placement of a coronary artery stent for progressive anginal symptoms. His coronary artery disease had predated his enrollment on study and was not felt by his cardiologist to be related to his participation on the trial.

Patient #3 was a 72-year-old man diagnosed in May 1998 with extensive non-small cell lung cancer. He refused standard chemotherapy and was started on dose level 1 SU5416 (65 mg/m^2 weekly dose) on October 12, 1998. He had stable disease by chest CT scan at his 12-week evaluation. Soon after this evaluation, he developed severe viral pneumonia resulting in a 2-week hospitalization and cessation of SU5416 therapy for 2 months. After being tapered from his steroids, he restarted therapy with SU5416 at dose level 2 (85 mg/m^2 weekly dose) but was removed from study after 6 weeks secondary to progressive disease as measured on his chest CT scan. His overall duration on study was 7 months.

Patient #6 was a 58-year-old man diagnosed in October 1995, with a left hilar squamous cell lung cancer. He was initially treated with chemotherapy including mitomycin C, vinblastine, and cisplatinum, followed by radiation therapy. In January 1998, his disease recurred in the left upper lobe (biopsy-proven). He received an additional six cycles of chemotherapy with carboplatinum and docetaxel. Nine months later his chest CT revealed progressive disease, and he was started on SU5416 at dose level 2 (85 mg/m^2 weekly dose). At week 12, his disease was stable by CT scan and he was continued on study. Two months later he developed an increasing pleural effusion and marked fatigue, and was removed from study. His duration on protocol was 5.5 months.

Of the remaining 15 patients who were evaluable for clinical responses, 9 were removed after a single cycle secondary to progressive disease (median duration on study equal to 6 weeks). Twelve of these patients have died from progressive disease. The 4 patients who obtained clinical benefit from participation on this trial remained on protocol between 8 and 48 weeks; 2 patients are currently alive.

Pharmacokinetic Analysis. After the 5-day loading dose, there is a 62% increase in clearance of SU5416 from the systemic circulation. When SU5416 infusions are given at an interval of 7 days, induction of clearance disappears, and clearance returns to baseline (Fig. 2). As demonstrated in Fig. 3, the percentage increase in clearance on daily dosing is similar to the induction of clearance (69%) observed when SU5416 is administered chronically via twice weekly dosing (Ref. 28; Fig. 3). An apparent difference in clearance in males (51.9 liter/h) and females (43.9 liter/h) is eliminated when clearance is corrected

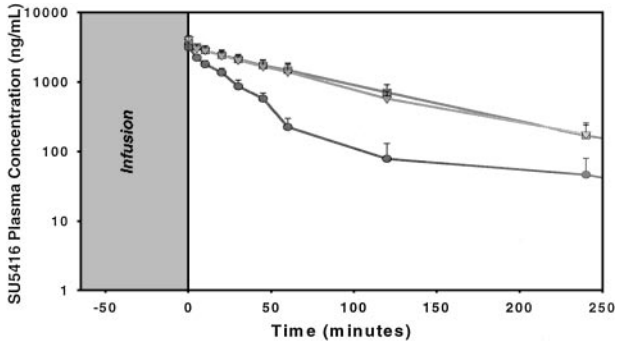

Fig. 3 All of the patients received 145 mg/m^2 SU5416 using either a weekly or biweekly infusion schedule. Plasma concentration of SU5416 on day 1 (■) and on day 25 (●) using the biweekly infusion schedule demonstrate induction of clearance, data from previous study (28). Plasma concentration of SU5416 on day 35 (▼) using the weekly infusion schedule shows a return to preinduction plasma concentrations; *bars*, ±SD.

for body surface area: males 25.9 and females 25.7 liter/h/m^2. There is no difference in total systemic clearance between elderly patients (those > or < 65 years old).

Distributive volumes of SU5416 correspond to the whole body fluid compartment and do not change materially over the treatment cycle. There is a short distribution (α) half-life followed by an elimination (β) half-life that varies from 21 to 87 min after the first infusion and from 18 to 66 min after the end of the loading dose. Similar to the change in clearance on the switch to weekly dosing, the elimination half-life returns to values observed on day 1 when the drug is administered weekly.

At the end of the infusion, plasma concentrations of SU5416 vary with dose administered, typically 0.8–2.4 μg/ml after the loading doses (20–65 mg/m^2) and 3.2–5.6 μg/ml after the maintenance weekly dose (85–190 mg/m^2). These plasma drug levels are sufficiently high to ensure that the concentration of SU5416 in plasma is sustained for 2 h at levels greater than that required for durable inhibition of human endothelial cell proliferation *in vitro* (29). AUCs appear to increase linearly with dose, although the number of patients in each of the five dose cohorts is small and there is a high interpatient variation in individual AUCs. One patient (#16, a male diagnosed with vascular sarcoma who experienced prolonged stabilization of disease with SU5416 treatment) had an AUC twice that of the next highest patient treated at the same dose level, with significantly prolonged exposure to the drug.

Angiogenic Markers. All of the patients collected 24-h urine specimens before initiating therapy, receiving their fifth daily dose of SU5416 during the 5-day loading period and on day 36 after receiving five weekly maintenance doses. Total 24-h quantitation of urine VEGF and bFGF was performed by ELISA assay. In a subset of 10 patients, the first voided urine was separated from the 24-h collection, and the amount of angiogenic proteins was quantified separately. As the ng/ml quantity of VEGF and bFGF from the initial void did not correlate with the 24-h collection, analysis of single-voided specimen was discontinued, and only total 24-h quantitation was used for analysis (data not shown). Urinary secretion of VEGF and bFGF levels varied 1–2-fold during the course of therapy but did not correlate with response or treatment dose (data not shown). Prestudy 24-h urinary secretion of VEGF and bFGF was compared in the 4 patients with clinical benefit (patients #3, #6, #14, and #16) to the remaining group of 18 patients. A statistically significant increase in the 24-h urinary levels of VEGF was found in the responding patients compared with the nonresponders (Table 5). There was no difference in the urinary levels of bFGF between these two groups of patients.

Table 5 Prestudy 24-h urinary levels of VEGF and bFGF

Total ng/24 h	Responders (n = 4 patients)	Nonresponders (n = 18 patients)
VEGF level		
Mean	323.4	182.7[a]
Range	164.6–510.9	37.9–465.1
bFGF		
Mean	4.20	4.63
Range	0.98–10.07	1.44–13.73

[a] $P < 0.05$.

DISCUSSION

More than 650 patients have been treated with SU5416 on a twice weekly i.v. schedule (25, 26, 30–32). All of the reported clinical data to date support a dose of 145 mg/m^2, as this dose is acceptable for chronic administration and is believed to provide a similar exposure to what was efficacious in preclinical models. To evaluate a less frequent dosing regimen that had showed antitumor potential in preclinical models, we initiated this trial testing a new schedule consisting of a 5-day loading period followed by weekly dosing. The highest dose used in this study was similar to that used in the twice-weekly schedule. A similar cluster of toxicities (headache with vomiting and nausea) was observed compared with the twice-weekly schedule. Thromboembolic disease was not observed in our study, suggesting either less endothelial cell activation or damage with once-weekly SU5416 dosing, or effective prophylaxis with low dose coumadin.

Drug pharmacokinetics were significantly different between the weekly and twice-weekly schedule (Fig. 3). With twice-weekly dosing, a 50–60% induction in clearance of SU5416 is observed with chronic dosing. Systemic exposure to the drug decreases after the initial week of therapy and plateaus thereafter, resulting in levels that are equivalent to what are used in animal and *in vitro* testing to produce biological activity. With the dosing schedule used in this trial, a similarly large induction in SU5416 clearance was observed during the 5-day loading period, but this induction reversed on weekly dosing. Patients receiving a weekly dose of SU5416 at 145 mg/m^2 had a consistently higher systemic exposure than those receiving the same dose on the twice-weekly schedule. This may explain the clinical observation that patients on the twice-weekly regimen of SU5416 appear to develop tachyphylaxis to the dose limiting toxicities. Conversely, those patients receiving weekly SU5416 infusions continued to experience these toxicities as long as they received the drug, consistent with higher drug levels being maintained throughout therapy.

The mechanism for induction in SU5416 clearance is not known but may be secondary to induction of liver enzymes caused by the drug, one of its metabolites, or the steroid premedication. *In vitro* evidence for induction of the cytochrome, CYP1A1, by SU5416 has been demonstrated; additional P450 cytochromes may also be involved in its biotransformation (33). In addition, induction of a drug efflux transporter may occur that effectively removes the drug from the systemic circulation.

Tumor-induced angiogenesis is a relatively new target for antitumor therapies; meaningful endpoints and/or surrogate markers of biological activity are still lacking (reviewed in Refs. 34, 35). SU5416 targets the VEGF pathway of tumor-induced angiogenesis. Many other tumor-induced or secreted growth factors are also known to foster angiogenesis; VEGF may only be necessary early in tumor progression (36, 37). Tumor growth that is initially dependent on VEGF stimulation may become independent of VEGF after reaching a critical size (38). In general, the patients treated on this trial had large tumor burdens and, thus, may already have been beyond the time when VEGF stimulation is necessary for tumor growth. The lack of clinical responses may also be related to inadequate systemic exposure in patients enrolled in the lower cohorts of this dose escalation trial.

Traditional measures of the antitumor activity of a drug (*i.e.*, radiographically measurable tumor regression) may not be an attainable end point when treating advanced malignancies with a drug targeting a single receptor signaling pathway. Disease stabilization or time to progression may be a more meaningful end point for this class of agents, a difficult end point to assess in Phase I trials with a heterogeneous mix of advanced cancer patients. Only one report of an animal model using SU5416 described significant regressions in established tumors (15). The vast majority of preclinical animal data obtained with single-agent SU5416 shows inhibition of tumor growth, with viable tumors still able to regrow on cessation of SU5416 therapy (27, 29). Of the 4 patients who gained clinical benefit in this trial, 3 patients obtained prolonged disease stabilization (>11 months for 1 patient), and only 1 patient obtained a true partial remission as measured by radiographic imaging.

Plasma levels of angiogenic factors, including VEGF and bFGF, have not been shown to be predictive of clinical responses in prior antiangiogenic trials (28). Plasma VEGF levels may be affected by multiple factors including menstrual cycle, hypoxia, smoking, platelet count, and progestins (39–43). Thus, we chose to measure the 24-h urinary secretion of VEGF in the hope that a longer window of observation would more accurately quantify angiogenic activity compared with a single plasma or serum level determination. Interestingly, we found that the prestudy 24-h urinary secretion of VEGF, but not bFGF, was significantly higher in the 4 patients who derived clinical benefit from SU5416 therapy. An elevated urinary level of VEGF may suggest a tumor that is dependent on VEGF stimulation for angiogenesis, neovessel stabilization, or even autocrine growth as described recently for both hematopoietic and solid tumors (44–49). Thus, urinary levels of angiogenic factors may prove to be useful in directing therapy against the most appropriate angiogenic target. Predictors of antitumor response have become extremely important in the selection of optimal therapy for a wide variety of tumors including breast cancer (estrogen and progesterone receptors, HER-2/*neu* status), lymphoma (CD20 expression), and acute myelogenous leukemia (CD33 expression). With recent studies revealing that tumor cells also express the VEGF receptors, Flt-1 and Flk-1/KDR, immunohistochemical analysis of tumor biopsies may provide additional predictive information in determining which patients are most likely to respond to a specific antiangiogenic therapy. Patients whose tumors express VEGF receptors may be ideal candidates for this therapy alone whereas patients whose tumors express multiple inducers of angiogenesis may require a combination of antiangiogenic agents or the addition of cytotoxic chemotherapy agents for significant tumor regressions. With the apparent redundancy in inducers and inhibitors of tumor-induced angiogenesis, methods to predict tumor responsiveness to therapies targeting specific pathways may save patients unnecessary toxicities and prove cost-effective in the long run.

The current dogma is that antiangiogenic therapies should be cytostatic rather than cytocidal. By targeting a different cellular pathway without the typical myelosuppressive toxicities of chemotherapy, antiangiogenic therapies are ideally suited for combining with standard cytotoxic agents. Many purported antiangiogenic therapies are already in Phase III clinical trials combined with standard chemotherapy regimens (31, 32, 50, 51). The manipulation of growth factor signaling may potentiate the efficacy of chemotherapy as suggested by clinical trials combining chemotherapy with trastuzumab (Herceptin) and animal models combining antiangiogenic agents with cytotoxics (52–54).

The exact role of antiangiogenic agents in the treatment of cancer patients is evolving. Methods to predict responders, safely and efficaciously combine antiangiogenic therapies with cytotoxic chemotherapies, and accurately monitor the biological activity of these agents are still areas to be explored. Our trial suggests that SU5416 is safe as a weekly infusion at a dose of 145 mg/m^2 and that weekly dosing maintains a comparatively higher systemic exposure for a given dose of SU5416 (*i.e.*, prevents the induction in clearance seen with twice-weekly infusions). Our results also suggest that biological efficacy may be maintained on a weekly schedule and that monitoring of urinary levels of angiogenic factors may prove helpful in predicting patients most likely to respond to VEGF signal transduction inhibitors.

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