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ORIGINAL ARTICLE

A phase II, pharmacokinetic, and biologic study of semaxanib and thalidomide in patients with metastatic melanoma

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Abstract *Purpose*: This phase II study evaluated the combination of semaxanib, a small molecule tyrosine kinase inhibitor of vascular endothelial growth factor (VEGF) receptor-2, and thalidomide in patients with metastatic melanoma to assess the efficacy, tolerability, pharmacokinetic (PK) and pharmacodynamic (PD) characteristics of the combination. *Patients and methods*: Patients with metastatic melanoma, who had failed at least one prior biologic and/or chemotherapeutic regimen, were treated with escalating doses of thalidomide combined with a fixed dose of semaxanib. *Results*: Twelve patients were enrolled and received 44 courses of semaxanib at the fixed dose of 145 mg/m^2 intravenously twice-weekly in combination with thalidomide, commencing at 200 mg daily with intrapatient dose escalation as tolerated. Treatment with semaxanib was initiated 1 day before thalidomide in the first course, permitting the assessment of the PKs of semaxanib alone (course 1) and in combination with thalidomide (course 2). The principal toxicities included deep venous thrombosis, headache, and lower extremity edema. Of ten patients evaluable for response, one complete response lasting 20 months and one partial response lasting 12 months were observed. Additionally, four patients had stable disease lasting from 2 to 10 months. The PKs of semaxanib were characterized by drug exposure parameters comparable to those observed in single-agent phase II studies, indicating the absence of major drug–drug interactions. Maximum semaximib plasma concentration values were 1.2–3.8 μg/ml in course 1 and 1.1–3.9 μg/ml in course 2. The mean terminal half-life was 1.3 (± 0.31) h. Biological studies revealed increasing serum VEGF concentrations following treatment in patients remaining on study for more than 4 months. *Conclusion*: The combination of semaxanib and thalidomide was feasible and demonstrated anti-tumor activity in patients with metastatic melanoma who had failed prior therapy. Further evaluations of therapeutic strategies that target multiple angiogenesis pathways may be warranted in patients with advanced melanoma and other malignancies.

Keywords Angiogenesis · Melanoma · Thalidomide · SU5416 · Phase II

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Introduction

Malignant melanoma is a disease with continuously increasing incidence and uniformly fatal outcome in the metastatic stage, accounting for 54,000 new cases and 7,600 deaths in the USA in 2003 [1, 2]. Immunotherapy

is a therapeutic option for high-risk melanoma in the adjuvant setting as well as for metastatic disease. Chemotherapeutic agents yield low response rates (approaching 15%) and no meaningful impact on patient survival [3–5]. Therefore, evaluating new therapies for advanced melanoma is a priority. Melanoma is a disease which lends itself to consideration of anti-angiogenic therapy because of a number of clinical and molecular features. A number of studies have shown that increased angiogenesis in melanoma and specifically the increased expression of vascular endothelial growth factor (VEGF) is associated with metastasis and poor prognosis [6, 7]. Moreover, increased serum concentration of angiogenic factors including VEGF relates to tumor progression and survival in patients with malignant melanoma [8]. In vivo studies demonstrated that the inhibition of VEGF production or activity has anti-tumor effect in several xenograft models including melanoma [9, 10]. Therefore, targeting relevant components of the angiogenic process is a rational therapeutic strategy in metastatic melanoma.

Thalidomide [α-(*N*-phthamido) glutarimide, Thalidomid™ (Celgene Inc., Summitt, NJ, USA)] is a glutamic acid derivate, originally marketed as a sedative. Approximately three decades later it was established that thalidomide has anti-angiogenic, immunomodulatory and anti-inflammatory properties that could contribute to its anti-tumor activity [11, 12]. It is now recognized that the mechanism of action of thalidomide is complex and involves downregulation of angiogenic cytokines and growth-promoting factors, including tumor necrosis factor alpha (TNFα), interleukin-6 (IL-6), VEGF, and basic fibroblast growth factor [13]. Additionally, recent studies suggest that thalidomide also blocks the activation of nuclear factor-κB and downregulates surface adhesion molecules in preclinical models [14, 15].

Nevertheless, specific molecular targets for thalidomide have yet to be elucidated. Thalidomide has demonstrated anti-tumor activity in patients with refractory multiple myeloma and has an established therapeutic role in this disease [16–18]. Encouraging results with thalidomide administered as a single-agent were also observed in myelodysplasia, Kaposi's sarcoma, renal cell carcinoma, and gliomas [19–21]. The toxicity profile of thalidomide in clinical trials included somnolence, skin rash, sensory neuropathy, drowsiness, and xerostomia as the main side effects [20–23]. Results of a phase II study of thalidomide in patients with advanced melanoma have been recently reported [21]. No objective responses were observed but 7 of 20 patients experienced disease stabilization for 12–32 weeks [22]. The combination of thalidomide with temozolomide has also been explored in patients with malignant melanoma. Five patients among the 12 enrolled experienced objective responses in the dose-finding study, with a response rate in the phase II study of 32% [22–24]. Additionally, a randomized phase II study compared the combination of temozolomide with either IFNα2b or thalidomide in patients with metastatic malignant melanoma [25]. The patients treated with the combination of temozolomide and thalidomide had a median survival of 7.3 months and a 1-year survival of 24% and therefore the combination was considered suitable for exploration in future studies. Although thalidomide administered as a single-agent did not result in major tumor regressions in malignant melanoma, the combination of thalidomide with cytotoxic or biological therapies including DTIC, IFNα2b, and temozolomide may result in increased anti-tumor activity and is therefore a promising approach for the treatment of malignant melanoma [26–28].

Semaxanib™ (SU5416, Sugen Inc., Redwood City, CA, USA) is a small molecule exhibiting potent and selective inhibition of VEGF receptor-2 (VEGFR-2, Flk-1/kinase insert domain receptor) tyrosine kinase [28]. Semaxanib binds more avidly to VEGFR-2 than the natural substrate, as shown by an inhibition constant (K_i) value of 0.16 μM, which is 3.3-fold lower than the Michaelis constant (K_m) of the kinase for ATP ($K_m = 0.53$ μM). It also inhibits the platelet-derived growth factor receptor tyrosine kinase with a $K_i = 0.57$ μM. Furthermore, semaxanib inhibits VEGF-stimulated tyrosine phosphorylation of Flk-1 ($IC_{50} = 1$ μM) in endothelial cells overexpressing Flk-1 receptors [29]. In vivo, semaxanib inhibits the growth of various subcutaneous xenografts in athymic mice, including A375 melanoma, C6 glioma, Calu-6 lung, A431 epidermoid, and LNCAP prostate carcinomas [30–33]. In phase I studies, the maximum tolerated dose of semaxanib as a single-agent was established as 145 mg/m^2 intravenously (IV) on a twice-weekly schedule, with the main side effects including headache, deep venous thrombosis, and arthralgias [34]. In a phase II study undertaken for patients with advanced melanoma, semaxanib appeared to be well tolerated and moderately effective [35]. Of a population of 26 patients, 1 experienced a partial response (PR), 1 stable disease (SD), and 5 had a mixed response. However, the low therapeutic index, inconvenient administration schedule, and the need for chronic corticosteroid therapy precluded further development of semaxanib.

Given the mechanism of action of semaxanib and thalidomide, it was hypothesized that the combination may result in robust inhibition in the signaling through the

VEGF pathway by antagonizing both VEGF production and VEGFR activation. In addition, the rationale for the combination of semaxanib and thalidomide was based on the potential for additive or synergistic anti-tumor activity and also on the non-overlapping toxicity profile of the two agents. The objectives of this phase II study were: (1) to determine the efficacy of the combination of escalating doses of thalidomide administered orally once daily with a fixed dose of semaxanib IV twice-weekly; (2) to characterize the principal toxicities of the combination; (3) to describe the pharmacokinetic (PK) behavior of semaxanib; and (4) to characterize biologic surrogates for anti-tumor activity.

Patients and methods

Patient selection

Patients with metastatic melanoma (proven cytologically or histologically), who had failed prior therapy were enrolled in the study. The eligibility criteria also included: (1) age > 18 years; (2) Eastern Cooperative Oncology Group performance status 0–1; (3) a minimum life expectancy of 12 weeks; (4) treatment with no more than one prior biologic and/or chemotherapy regimen; (5) no major surgery within 14 days; (6) no prior large field radiation therapy (> 20% total bone marrow); (8) bidimensionally measurable disease; (9) adequate organ function including hematopoietic [absolute neutrophil count > 1,500/μl, platelets > 100,000/μl, hemoglobin > 8.5 g/dl], hepatic [bilirubin < 1.5 mg/dl, aspartate transaminase (AST) and alanine transaminase (ALT) < 2.5 times institutional upper normal limit], and renal functions (serum creatinine < 1.5 mg/dl or creatinine clearance > 60 ml/min); (10) coagulation tests within normal limits; (11) no active infection; (12) no severe gastrointestinal disturbance or major upper gastrointestinal surgery that could preclude oral administration or gastrointestinal absorption; (13) no diabetes mellitus with severe peripheral vascular disease or history of thrombosis; (14) no uncompensated coronary artery disease or a history of myocardial infarction or severe/unstable angina in the 6 months prior to study enrollment; and (15) no active neoplastic involvement of the central nervous system. All patients gave written informed consent before treatment according to federal and institutional guidelines.

Dosage and drug administration

This study was designed as an open label, phase II trial of the combination of semaxanib and thalidomide in patients with metastatic melanoma. Semaxanib was administered at a fixed dose of 145 mg/m^2 twice-weekly (day 1 and day 4) over 1 h IV via a central venous access device. All patients received premedication with diphenhydramine, famotidine, and dexamethasone prior to the semaxanib infusion. Thalidomide was commenced at 200 mg orally at bedtime, 1 day following the first dose of semaxanib thus permitting the evaluation of PK interactions. Intrapatient dose escalation of thalidomide occurred in 100–200 mg increments every 1–2 weeks (up to a total dose of 1,000 mg), according to individual tolerability. A course of therapy was arbitrarily defined as 4 weeks.

Semaxanib was supplied by the National Cancer Institute (NCI) as a parenteral yellow-orange, sterile, aqueous-insoluble solution, formulated in 50 ml vials containing 180 mg semaxanib in 40 ml of vehicle, for a final concentration of 4.5 mg/ml with the drug product containing polyoxyethylated castor oil (Cremophor). Prior to administration, semaxanib was diluted 1:3 with 0.45% sodium chloride. Administration sets were made from low absorption polyethylene tubing or polyethylene-lined tubing. Semaxanib was administered through an infusion set containing a 0.22-μm filter made from hydrophilic polyethylsulfone. Thalidomide was supplied by the NCI as 50 mg hard gelatin capsules.

Dose-limiting toxicity (DLT) was defined by the occurrence of grade 4 hematologic or grade 3 non-hematologic toxicity except for grade 2 peripheral neuropathy which was also considered dose limiting. Any DLT required holding the treatment until toxicity resolved to grade 1 and dose reduction for subsequent cycles. Patients were permitted to continue on study in the absence of disease progression regardless of the number of dose reductions or the time to recovery from toxicity, as long as toxicities resolved to grade 1. Adverse events were categorized using Common Toxicity Criteria Version 2.0, NCI.

Statistical considerations

The study utilized the Simon's optimal two-stage design [36]. The null hypothesis being that the overall response rate was to be $\leq 10\%$ vs the alternative hypothesis that the overall response rate was to be over 30%. In the first stage, 12 evaluable patients were to be enrolled. If no response was observed in the first 12 patients, the trial was to be terminated; otherwise an additional 23 patients were to be treated for a total of 35 patients. This design yielded ≥ 0.90 probability of a positive result if the true response rate was $\geq 30\%$. It

yielded a ≥ 0.90 probability of a negative result if the true response rate was ≤ 10% with a ≥ 0.65 probability of early negative stopping.

Pretreatment and follow-up studies

A complete medical history and physical examination and routine laboratory evaluations were performed pretreatment, weekly for the first 4 weeks and every 2 weeks thereafter. Routine laboratory evaluations included complete blood cell counts with white blood cell differential, electrolytes, creatinine, blood urea nitrogen, total protein, albumin, bilirubin, alkaline phosphatase, AST, ALT, glucose, uric acid, calcium, phosphate, clotting time, and urinanalysis. A corticotropin stimulation test was obtained pretreatment and then prior to each course to evaluate patients for adrenal responsiveness. In order to further evaluate the potential thrombogenic effects of semaxanib, extensive coagulation tests including fibrinogen, D-dimmers, protein C, protein S, and anti-thrombin III and factor V Leiden were performed pretreatment and during weeks 2 and 4 of course 1. Radiological studies for disease assessment were conducted pretreatment and following every second course. WHO (bidimensional) criteria were used for tumor evaluation. The patients were allowed to remain on study until their disease has progressed by ≥ 50%, in order to allow for a delayed anti-tumor effect in patients tolerating therapy well.

Pharmacokinetics

Semaxanib: plasma and urine sampling

Blood samples for semaxanib were collected on day 1 of the first and second course of treatment. Blood samples (5 ml) were collected in heparinized tubes predose and at 10, 35, and 45 min, 1, 1.5, 2, 4, 6, 8, and 24 h after the semaxanib infusion. Total urinary volume was collected from 0–8, 8–24, 24–48, and 48–72 h during the first week of course 1. Immediately after collection, all samples were centrifuged at 3,000 rpm for 15 min, transferred to labeled cryostorage tubes, and frozen at −80°C until analysis.

Analytical methodology

Concentrations of semaxanib in plasma were measured by high-pressure liquid chromatography utilizing a method developed and validated by Sugen Inc. All procedures have been adapted from a previously published method [37].

Pharmacokinetic analyses for semaxanib

Non-compartmental modeling and parameter estimation were performed using WinNonLin® (Pharsight Corporation, Mountain View, CA, USA). The area under the concentration–time curve from time zero to the time of the final quantifiable sample (AUC_{0-Tf}) was calculated using the linear trapezoid method [38]. The AUC was extrapolated to infinity (AUC_{0-inf}) by dividing the last measured concentration by the terminal rate constant (k), which was calculated as the slope of the log-linear terminal portion of the plasma concentration–time curve using linear regression. The terminal phase half-life ($t_{½}$) was calculated as $0.693/k$. The observed maximum plasma concentration (C_{max}) and the time to maximum concentration (T_{max}) were determined by inspection of the concentration–time curve.

Pharmacodynamics

Urine and serum samples for VEGF, TNFα, matrix metaloproteinases (MMP) MMP-1, MMP-2, and MMP-9 were collected prior to drug administration, weekly for the first course and on week 1 of the subsequent courses of the combination.

Methods for biological markers

Matrix metaloproteinases-2 ELISA kit was purchased from Amersham (Piscataway, NJ, USA); all other kits were obtained from R&D Systems (Minneapolis, MN, USA). Frozen plasma, serum, and urine specimens were thawed on ice and assayed in duplicate without dilution according to the kit supplier's instructions. In addition, for specimens with high levels of MMP-9, the assays were performed at 1:10 and 1:50 dilution. Final absorbances were read in a Tecan SPECTRA Fluor Plus multiwell (Tecan, Toronto, Canada) plate reader. The levels of MMP-1, MMP-2, MMP-9, VEGF, and TNFα were quantified by comparison with serial dilutions of respective standards. The detection ranges for the ELISAs were: MMP-1, 0.156–10 ng/ml; MMP-2, 1.5–24 ng/ml; MMP-9, 0.312–10 ng/ml; VEGF, 31.2–1,000 pg/ml; and TNFα, 15.6–500 pg/ml (4.4 pg/ml).

Results

General

A total of 12 patients were enrolled in this study, all evaluable for toxicity. Pertinent demographic charac-

teristics for the cohort as well as individual characteristics of each patient are illustrated in Tables 1 and 2. Only two patients underwent thalidomide dose escalation. One patient tolerated a dose escalation from 200 to 300 mg of thalidomide when combined with a full dosage of semaxanib. For a second patient according to his tolerance, the dosage of thalidomide was increased to 300 mg and subsequently to 400 mg while the semaxanib dosage was reduced to 110 mg/m^2 and further to 85 mg/m^2. The dose escalation scheme is depicted in Table 3.

Table 1 Patient characteristics

Characteristics	Number of patients
Total patients (evaluable for response)	12 (10)
Age median (range)	58 (43–71)
Male/female	6/6
Number of courses	
Total	44
Median per patient (range)	2 (1–14)
ECOG performance status	
0	4
1	7
2	1
Previous treatment	
Immunotherapy	4
Chemotherapy	10
Radiotherapy	5
None	0

ECOG Eastern Cooperative Oncology Group

Table 2 Patient characteristics

Patient	Age	Sex	Previous treatment	No. of cycles	Response
1	64	M	S, R	1	N/A
2	71	M	S, I, R	1	N/A
3	45	F	M, R	2	SD
4	71	F	I	14	PR
5	50	F	M	4	SD
6	49	M	M	1	PD
7	62	F	S	6	SD
8	66	M	M, R	2	PD
9	43	F	I	1	PD
10	59	F	S	4	SD
11	57	M	M	8	CR
12	50	M	M, I, R	1	PD

R radiotherapy, *S* single-agent chemotherapy, *I* immunotherapy, *M* multi-agent chemotherapy, *SD* stable disease, *PD* progressive disease, *PR* partial response, *CR* complete response

Non-hematological toxicities

The principal non-hematological toxicities of the regimen were headache in eight patients, that reached grade 3–4 in three patients and grade 3–4 thrombosis in three patients. Patients who experienced severe headache were all female, aged 43–59, with a history of migraine (two patients) or anxiety (one patient). This side effect occurred predominantly after the first infusion of semaxanib and decreased to a grade 1 or 2 with premedication using non-steroidal anti-inflammatory drugs for subsequent infusions. One patient, however, decided to withdraw consent after experiencing a grade 3 headache in course 1.

One patient experienced a pulmonary embolism on day 20 of the first course. Two additional patients experienced grade 3 thrombosis of the internal jugular vein and subclavian vein, respectively. For both these patients, no extension to the vena cava and no sign of pulmonary embolism was detected on a spiral computerized tomography scan. These thromboembolic events were considered drug related and affected patients were discontinued from study as required by the protocol. Sensory neuropathy, described as intermittent tingling and numbness mainly in the upper and lower extremities was observed in eight patients but generally mild to moderate: grade 1 for five patients and grade 2 for two patients. Only one patient experienced grade 3 sensory neuropathy after the first course. The same patient experienced additional neurological symptoms including headache, vertigo, loss of balance and was diagnosed with brain metastases. Lower extremity edema was a frequent but tolerable side effect (grade 1 for two patients and grade 2 for three patients). The severity of the edema appeared to increase with the number of courses of treatment received.

Table 3 Dose escalation scheme

Dose SU5614 (mg/m^2)/ thalidomide (mg/day)	New patients (courses)	Patients reduced to dose (courses)	Patients increased to dose (courses)
Dose level 1: 145/200	12 (41)	0 (0)	N/A
Dose level 2: 145/300	0	0 (0)	1 (5)
Dose level −1: 110/300	0	1 (1)	0 (0)
Dose level −2: 110/400	0	1 (2)	0 (0)
Dose level −3: 110/100	0	1 (1)	0 (0)
Dose level −4: 85/400	0	1 (4)	0 (0)

Other toxicities were mild to moderate (grade 1 or 2) and included: asthenia (11 patients), constipation (3 patients), hypercholesterolemia (1 patient), and hyperglycemia (2 patients). One patient experienced asymptomatic grade 4 hypertriglyceridemia after four courses of treatment and received atorvastatin calcium with significant lowering of triglyceride levels permitting continued study participation at a lower dose level. Two patients experienced asymptomatic grade 4 hyperglycemia related to the corticosteroid therapy required as premedication for semaxanib. Of note, no significant changes were observed in serum cortisol levels or coagulation tests performed during treatment; however only six patients completed these laboratory tests according to the protocol. Principal non-hematological toxicities are summarized in Table 4.

Hematological toxicity

Hematological toxicity was minimal and included grade 1 anemia in three patients. No neutropenia, lymphopenia, or thrombocytopenia was observed.

Anti-tumor activity

Ten patients were evaluable for anti-tumor activity. Therapy was discontinued for two patients before completion of one course and without tumor evaluation: one patient withdrew consent after 2 weeks for personal reasons in the absence of significant toxicity and one patient was found to have a second primary tumor (non-small cell lung cancer). Four patients received only one course of semaxanib and thalidomide. Of these, two patients experienced early progressive disease (PD) and two were discontinued for toxicity after one course. Tumor evaluation was, however, performed for these two patients and did not show evidence of progressive disease. Six patients completed at least two courses of the combination. Among these, one patient experienced a complete response (CR), one patient a PR, and four additional patients had SD lasting 2–10 months. A 57-year-old male experienced a CR for 20 months. The patient had axillary and supraclavicular lympadenopathy as well as lung metastases and had failed prior treatment with DTIC, vinblastine, and cisplatin. Tumor evaluation after four courses showed complete regression of supraclavicular lymph nodes and lung nodules and a major reduction of the axillary mass. After course 6, the patient developed an abscess in the axillary area and underwent surgical treatment. No gross residual tumor was found in the axilla during the surgery. After eight courses, the patient developed Cushingoid features and immunosupression, which were attributed to the corticocorticosteroid therapy required as premedication. The patient was discontinuated from the study following nine courses of treatment after developing cellulitis of the left arm. He maintained a CR for 12 months after treatment discontinuation. A 71-year-old female with lung and liver metastases, previously treated with adjuvant interferon experienced a PR for a duration of 12 months, as well as improvement in disease-related symptoms including cough, dyspnea, and pain.

Pharmacokinetics and pharmacodynamics

All 12 patients had plasma sampling performed for semaxanib in course 1, and 7 patients in course 2. The principal PK parameters for semaxanib are summarized in Table 5. C_{max} ranged from 1.2 to 3.8 μg/ml in course 1 and 1.1 to 3.9 μg/mL in course 2; $t_{1/2}$ was 1.3 (± 0.31) h. The PK parameters from the first and second courses were similar, suggesting neither major drug accumulation nor drug interactions. Additionally, PK parameters of exposure were similar to those observed in single-agent phase II studies, further supporting the conclusion of absence of drug interactions. Scatterplots of C_{max} and AUC values of semaxanib for

Table 4 Non-hematological toxicities

Adverse event	No. of events		
	All courses/all grades (1st course)	Grade 3 (1st course)	Grade 4 (1st course)
Headache	8 (4)	3 (2)	1 (1)
Thrombosis	4 (2)	3 (1)	1 (1)
Hypercholesterolemia	2 (0)	1 (0)	1 (0)
Hypertriglyceridemia	1 (0)	0	1 (0)
Neuropathy	5 (3)	1 (1)	0
Edema	5 (1)	1 (0)	0
Asthenia	11 (3)	0	0
Constipation	3 (2)	0	0

the seven patients having PK sampling on course 1 and course 2 are depicted in Figs. 1 and 2. Comparison of the C_{max} and AUC values between cycle 1 and 2 showed no significant differences over time for C_{max} but a decrease in the AUC values in cycle 2 (not statistically significant).

The levels of VEGF, TNFα, MMP-1, MMP-2, and MMP–9 were determined in plasma, serum, and urine samples. Serum, plasma, and urine samples are available from three patients and serum/urine samples from nine patients. Serum VEGF levels increased slightly over time in the five patients receiving more than four cycles of therapy, whereas serum VEGF levels decreased between cycle 1 and 2 in the five patients receiving less than 4 months of the combination. Urine TNF results were available only from three patients. The TNF levels decreased in one patient and rise in two others. Results are presented in Table 6. No significant differences in the VEGF levels at baseline, and after treatment have been noted for the two responders.

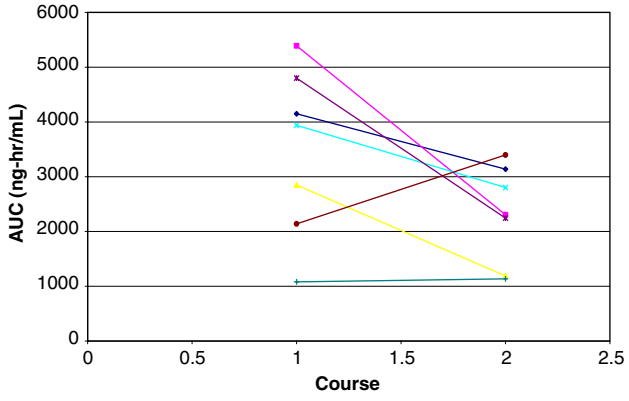

Fig. 1 Semaxanib AUC course 1 and 2 in seven patients

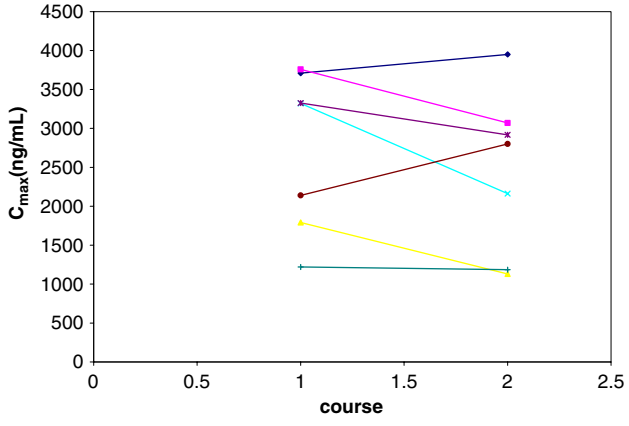

Fig. 2 Semaxanib C_{max} course 1 and 2 in seven patients

Table 5 SU5416 pharmacokinetic parameters

Parameters	Course 1 Mean value (SD)	Course 2 Mean value (SD)
$AUC_{[I]}$ (ng h/ml)	3,258.0 (1,463.5)	2,315.8 (889.8)
$AUC_{[tf]}$ (ng h/ml)	3,219.2 (1,464.6)	2,293.1 (889.5)
CL (ml/h)	114,849 (83,404)	131,593 (90,446.7)
V_{ss} (l)	82,162 (48,281.7)	76,016 (47,991.8)
$t_{1/2}$ z (h)	1.3 (0.31)	1.1 (0.42)
C_{max} (ng/ml)	2,647.1 (947.5)	2,459.3 (1,033.1)
T_{max} (h)	0.2 (0.096)	0.2 (0.128)

AUC area under the curve, *CL* clearance, V_{ss} volume of distribution at steady state, C_{max} maximum concentration, T_{max} time at the maximum concentration, *SD* standard deviation

Discussion

Angiogenesis, the process of forming new vasculature, was shown to be essential for tumor growth beyond 1–2 mm^3 and for the development of metastases [39, 40]. Tumor invasiveness can be enhanced by the recruitment of pro-angiogenic factors such as VEGF, PDGF, TGFα and β, IL-8 [41]. Among these, VEGF and its receptors play a major role in promoting malignant angiogenesis. Overexpression of VEGF has been associated with tumor progression and poor prognosis in several tumor systems, including melanoma, colon, gastric, pancreatic, breast, and prostate carcinomas [6, 42–50]. Based upon these findings, significant efforts to evaluate therapeutic compounds targeting VEGF signaling were undertaken. Given that anti-angiogenic therapies were intended to block the development of new blood vessels, it seemed unlikely that they would induce significant tumor regression in clinical studies but instead would potentially stabilize rapidly growing tumors through tumor growth inhibition. Therefore, the primary endpoints for treatment success were redefined to include inhibition of tumor growth instead of tumor regression. For many years, anti-angiogenic therapy has failed to fulfill its promise in the clinical setting, and more than 50 clinical trials with a variety of anti-angiogenic drugs have yielded disappointing results [51]. However, the recent approval of bevacizumab in combination with chemotherapy for the treatment of patients with metastatic colorectal carcinoma, based upon a survival advantage, strongly supports further research in this area [52]. One possible explanation for the failure of earlier trials with anti-angiogenic agents may be the complex nature of angiogenesis, involving multiple and often redundant pathways [33, 34], where blocking only one putative pathway is unlikely to result in robust anti-tumor effects. This hypothesis is supported by preclinical

Table 6 Pharmacodynamic parameters

Patients on study	VEGF (ng/ml) mean				MMP-1 (ng/ml)				MMP-2 (ng/ml)			
	C1 W1	C1 W2	C2 W1	C3 W1	C1 W1	C1 W2	C2 W1	C3 W1	C1 W1	C1 W2	C2 W1	C3 W1
>4 courses (five patients)	14.0	51.0	96.0	187	19.5	38.8	8.81	24.6	88.2	73.1	55.4	55.9
<4 courses (five patients)	76.0	146.0	27.0	30.0	5.1	20.8	23.0	N/A	40.0	34.7	17.8	N/A

VEGF vascular endothelial growth factor, *MMP* matrix metaloproteinases, *C* course, *W* week

studies where, combined therapies targeting different components of angiogenesis have shown enhanced anti-tumor effects [53]. Thus, combining the VEGFR-2 inhibitor semaxanib with an agent capable of inhibiting endothelial cell growth as well as the synthesis of pro-angiogenic cytokines such as thalidomide represented a rational therapeutic approach.

Although this study was discontinued prematurely by the NCI, based on Sugen's decision to pursue development of other second-generation VEGFR inhibitors, this trial nonetheless suggests the feasibility and efficacy of dual anti-angiogenic therapy, albeit in a small patient population. Generally, toxicities were those expected and described with semaxanib and thalidomide used as single-agents including headache, asthenia, constipation, lower extremity edema, neuropathy, hyperglycemia, hypercholesterolemia, and thrombosis [17, 31]. No significant lethargy was observed in the current study and hematological side effects were minimal. However, non-hematological side effects including thromboembolic events and headache mostly attributable to semaxanib, were prominent and in several patients precluded further treatment with the combination. One patient experienced a CR, one patient a PR, and four additional patients had SD resulting in tumor growth control (CR + PR + SD) in 50% of patients. In addition, the duration of tumor responses (20 and 12 months for the patient with CR and PR, respectively) is noteworthy although it is difficult to determine the significance of these results given the small patient numbers and the potential for more indolent disease in highly selected patients.

Pharmacokinetic results demonstrate semaxanib parameters of exposure comparable to those observed in single-agent phase I studies, suggesting the lack of major drug interactions between semaxanib and thalidomide. Comparison of the C_{max} and AUC values between course 1 and 2 showed no significant differences for C_{max} but a decrease in the AUC values in course 2. This is consistent with results from other studies reporting an induction of clearance of 50–60% for semaxanib on the daily or biweekly dosing schedule [33, 34]. The mechanism of the increase in clearance is not known but may be secondary to liver enzyme induction either by the drug or by the corticosteroids required for premedication. The PD analysis revealed a trend toward an increase in serum VEGF levels for the patients receiving more than four cycles of treatment. However, no statistical comparisons could be performed in this small patient population. This observation appears consistent with recent studies suggesting that high urinary and plasma levels of VEGF may correlate with clinical response to a VEGFR inhibitor [31, 33]. One possible explanation for these findings may be the decrease of internalized VEGF following receptor binding during the treatment with a VEGFR inhibitor thus leading to an increase in circulating VEGF levels [31, 33].

The current study is among the first to explore the feasibility of dual anti-angiogenic therapy. This trial was designed before the established therapeutic efficacy of angiogenesis inhibitors and thus represented a highly novel and innovative concept. The anti-tumor activity observed in this study suggests that metastatic melanoma may be dependent on aberrant angiogenesis and that abrogation of multiple pathways may thus be a successful therapeutic approach. Although semaxanib development was discontinued, novel anti-angiogenesis agents such as SU11248 (Sugen Inc.) and Sorafenib (Bayer Pharmaceuticals Corporation, West Haven, CT, USA) have recently garnered FDA approval and other agents such as SU6668, PTK787 (Novartis, Pharma AG, Basel, Switzerland), and ZD6474 (Astra-Zeneca, Pharmaceuticals LP, Willington, DE, USA) are currently in clinical development and are of particular interest for combination studies due to their favorable toxicity profile and potential for chronic administration.

In conclusion, the current study is of importance as it provides a platform for considering future combinations of anti-angiogenic agents in patients with malignant melanoma and other solid tumors.

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