Phase II Study of the Flk-1 Tyrosine Kinase Inhibitor SU5416 in Advanced Melanoma

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ABSTRACT

Purpose: Vascular endothelial growth factor (VEGF) expression is prognostic in melanoma, and the activity of VEGF is mediated in part through the receptor tyrosine kinase Flk-1. A Phase II study of SU5416, a preferential inhibitor of Flk-1, was carried out in patients with metastatic melanoma to determine clinical response, tolerability, and changes in tumor vascular perfusion.

Experimental Design: Patients with documented progressive disease and ≤1 prior therapy were eligible. Central nervous system metastases were allowed if stable off medication. SU5416 (145 mg/m²) was administered via a central catheter twice weekly for 8 weeks. Premedication with dexamethasone, diphenhydramine, and a H₂ blocker was required because of the Cremophor vehicle. Tumor vascular perfusion was assessed before treatment and during week 8 by dynamic contrast magnetic resonance imaging, and plasma was analyzed for VEGF.

Results: Thirty-one patients were enrolled. Two-thirds had received prior therapy, 21 had visceral metastasis, and 14 had an elevated lactate dehydrogenase. Mean absolute lymphocyte counts were decreased (P = 0.002), and glucose levels were increased (P = 0.001) posttherapy, presumably because of steroid premedication. Four vascular adverse events were observed. Of 26 evaluable patients, 1 experienced a partial response, 1 had stable disease, and 5 had a mixed response. Dynamic contrast magnetic resonance imaging in 5 evaluable patients showed decreased tumor perfusion at week 8 (P = 0.024), and plasma VEGF levels were elevated compared with pretherapy (P = 0.008).

Conclusions: SU5416 appears to be relatively well tolerated in this population. Although the modest clinical activity and potential effects on tumor vascularity may support additional exploration of VEGF as a target in melanoma, effects from steroid premedication limit further investigation of this agent.

INTRODUCTION

Angiogenic factors released from tumor cells and/or infiltrating stromal cells recruit and activate vascular endothelial cells, leading ultimately to new blood vessel formation. This process appears to be required for solid tumors to grow beyond a volume of 2 mm³ (1). Solid tumors that lack an adequate vasculature become necrotic and/or apoptotic (2, 3), whereas tumors that have undergone neovascularization can enter a phase of rapid growth and may demonstrate increased metastatic potential. Numerous studies have correlated increased tumor blood vessel density with poor prognosis in patients with various malignancies, including melanoma (4, 5).

Melanoma is a highly vascular tumor, suggesting that strategies to target angiogenesis are theoretically attractive. Expression of one angiogenic molecule, vascular endothelial growth factor (VEGF), has been shown to be prognostic for melanoma metastasis (6, 7). In mouse preclinical models, strategies that inhibit VEGF production or activity are therapeutic in vivo (8–11). Collectively, these results indicate that interference with VEGF activity in melanoma may provide a strategy to control tumor growth.

There are multiple receptors for VEGF expressed by endothelial cells, including Flk-1, Flt-1, Tie-1, and Tie-2 (12, 13). Several preclinical studies have addressed the relative importance of these receptors in tumorigenesis (14, 15). Flk-1 is a receptor tyrosine kinase that is thought to play a critical role in VEGF-mediated angiogenesis and blocking Flk-1 activity in murine xenograft models has been shown to result in tumor regression (16). SU5416 is a potent inhibitor of the tyrosine kinase activity of Flk-1 that has been shown to inhibit VEGF-dependent proliferation in animal models (17). Phase I clinical studies of SU5416 have examined two schedules, one consisting of a 5-day loading dose followed by weekly i.v. infusions and the other consisting of biweekly i.v. infusions. In both schedules, a dose of 145 mg/m² was well tolerated and recommended for additional testing (18–20). Clinical responses and disease stabilization were also observed in a subset of patients. On the basis of these preclinical and Phase I clinical trial data, we undertook a Phase II study to investigate the activity of SU5416 in patients with documented progressive metastatic melanoma. Specifically, we wished to address clinical response, the tolerability of this drug, and its effects on tumor vasculature as measured by dynamic contrast magnetic resonance imaging (MRI; Ref. 21).

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PATIENTS AND METHODS

Eligibility. This was an open label, multi-institutional Phase II trial of SU5416 in patients with advanced melanoma approved by the University of Chicago Institutional Review Board. The study drug was provided by the Cancer Treatment Evaluation Program of the National Cancer Institute. Adult patients ≥ 18 years of age with histologically confirmed melanoma and documented measurable and progressing metastases (including nonresectable in transit metastases) were allowed if they had ≤1 prior therapy with an interval ≥ 4 weeks from the previous therapy. Patients with brain metastasis were allowed if stable off steroids and anticonvulsants. Additional inclusion criteria included life expectancy of ≥12 weeks, WHO performance status of ≤2, and adequate organ function (defined as WBC ≥ 3000/μl, platelets ≥ 100,000/μl, total bilirubin ≤ 1.5 mg/dl, transaminases ≤ 2.5× upper limit of normal, and serum creatinine ≤ 1.5 mg/dl or CrCl ≥ 60 ml/min).

Patients with any of the following findings were excluded: uncompensated coronary artery disease; myocardial infarction or severe/unsafe angina within the previous 6 months; diabetes mellitus with severe peripheral neuropathy; history of deep venous thrombosis or arterial thrombosis within 3 months of entry; a history of another malignancy within the past 5 years (except curatively treated nonmelanoma skin cancer or carcinoma in situ of the cervix); pregnancy or unwillingness to use contraception while on study; any uncontrolled medical condition; or any psychiatric illness that would interfere with patient compliance or informed consent. Also excluded were patients with a history of severe allergic or anaphylactic reactions to paclitaxel or docetaxel. All patients gave written informed consent before enrolling in the study.

Treatment Plan. All patients were required to have a central venous catheter for drug administration and were placed on 1 mg/day oral coumadin for thrombosis prophylaxis. SU5416 was formulated in a Cremophor vehicle, necessitating premedication against idiosyncratic anaphylaxis as is done for taxanes. Patients received oral dexamethasone (10 mg) at 12 and 6 h before the first dose of SU5416 or if therapy had been interrupted for any period of time. Subsequent dexamethasone doses were reduced to 4 mg at the same schedule if tolerated. In addition, diphenhydramine (25–50 mg) and famotidine (20 mg) were administered i.v. before each SU5416 dose. One part SU5416 (4.5 mg/ml) was diluted by adding two parts normal saline and infused at a dose of 125 mg/m² at a rate of 100 ml/h for the first 15 min, then increased to 200 cc/h. Patients were observed in the chemotherapy suite for 3 h after their first, second, and third doses of SU5416. For subsequent doses, the observation time was reduced to 30 min. Vital signs were recorded every 15 min. One cycle consisted of 4 weeks of biweekly infusions.

Clinical response was assessed every two cycles. Patients were withdrawn from the study if they showed disease progression, if >4 weeks elapsed between treatments for any reason, if there were intolerable adverse events, or if the patient chose to be withdrawn. Dynamic contrast MRI and measurement of plasma VEGF levels were performed before and upon completion of 8 weeks of therapy in patients when possible.

Evaluation of Response and Toxicity. Toxicity assessments according to the National Cancer Institute Common Toxicity Criteria Scale were performed every 4 weeks or at the beginning of each cycle. Clinical and imaging responses were recorded after two cycles of treatment and then at 8-week intervals. Response Evaluation Criteria in Solid Tumors criteria were used to assess clinical response. Mixed responses, i.e., shrinkage of at least one tumor despite progressive growth of
another tumor were documented but formally recorded as progressive disease. Nonevaluable patients were those removed from study before the next scheduled evaluation for reasons not related to obvious disease progression.

**Dynamic Contrast MRI.** For the dynamic contrast MRI, one of the metastases was required to be in or near the liver to allow for comparison to this normal reference tissue at each time point as an internal control. Dynamic MRI of tumors was obtained in patients before treatment and upon completion of 8 weeks of therapy. To simplify the computational difficulties, we chose to examine semiquantitative parameters. The peak concentration of contrast agent in the tumor as well as the peak concentration in normal liver were obtained, and a ratio was recorded for each assessment.

Images were acquired using SIGNA 1.5 Tesla scanners (General Electric Medical Systems, Waukesha, WI) equipped with self-shielded (Echo Speed+) gradients that allow rapid imaging without eddy current distortion. One to four slices were selected through lesions and surrounding normal tissue. These slices were imaged with time resolution between 1 s (when one slice was selected) and 4 s (when four slices were selected). The images were acquired using a spoiled gradient pulse sequence with evolution time (TE) of 1.7 ms, repetition time (TR) of 8 ms, and flip angle of 60°. The matrix size was 128 × 256 in the phase and frequency encoding directions, respectively. Field of view was typically 40 cm.

**MRI Data Analysis.** To obtain results that were independent of instrumental parameters and allowed quantitative analysis, the raw data were used to calculate concentration of contrast agent C(t) as a function of time after contrast injection, based on estimates of the change in T1. To save time in the clinical setting, we estimated ΔT1 and C(t) based on comparison of the signal in the tumor with the control signal in a reference tissue (normal liver) with known T1. In our heavily T1-weighted images, the signal is approximately inversely proportional to the T1 of the tissue. Thus, when both the precontrast signal intensity and the native T1 in the reference tissue are known, the change in T1 in each image pixel after contrast injection can be calculated from the change in signal intensity. The concentration of contrast media as a function of time was calculated directly from the change in T1 based on the relaxivity of the contrast agent, which is constant at a field strength of 1.5 T.

**Plasma Measurement of VEGF Levels.** Approximately 10 ml of blood were collected into heparinized tubes before the first dose of SU5416 and during week 8. Plasma was isolated by centrifugation, and samples were cryopreserved until analysis. Measurement of VEGF content was performed using anti-VEGF antibody pairs from R&D Systems (Minneapolis, MN) and standard ELISA methods. The sensitivity of the assay was ~5 pg/ml.

**Statistical Considerations.** Time to progression and overall survival estimates were calculated using the Kaplan-Meier method. For assays of serum glucose, lymphocyte counts, and VEGF levels pre- and posttherapy, unpaired t tests were used; for the dynamic MRI assessments, paired t tests were used. Statistical calculations were performed using SPSS software.

**RESULTS**

**Patient Characteristics.** Thirty-one patients were enrolled, and their characteristics are summarized in Table 1. Two-thirds of the patients had received prior therapy, and only

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**Table 3 Clinical response**

<table>
<thead>
<tr>
<th>Response</th>
<th>No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>PR(\text{a})</td>
<td>1</td>
</tr>
<tr>
<td>SD</td>
<td>1</td>
</tr>
<tr>
<td>PD</td>
<td>19</td>
</tr>
<tr>
<td>MxR(\text{b})</td>
<td>5</td>
</tr>
<tr>
<td>Not evaluable</td>
<td>5</td>
</tr>
</tbody>
</table>

\(\text{a}\) PR, partial response; SD, stable disease; PD, progressive disease; MxR, mixed response.

\(\text{b}\) Mixed response, as defined by regression of one or more lesions with progression of other lesions.
one-third of the patients had nonvisceral metastatic disease. Approximately one-half of the patients had an elevated serum lactate dehydrogenase, which is an important negative prognostic factor in melanoma. Four patients had documented central nervous system metastasis before study entry. As a prerequisite for study participation, all patients had documented progressive disease before enrolling.

**Adverse Events and Toxicities.** The common and notable toxicities are listed in Table 2. SU5416 was generally well tolerated in this patient population. There were three dose reductions, two because of grade 3 nausea/vomiting and one because of anemia. Of note, lymphopenia and hyperglycemia were commonly observed, presumably due to the steroid premedication. As shown in Fig. 1, the mean absolute lymphocyte count decreased from 1700 to 400/μl (P = 0.002), and the mean glucose level increased from 110 mg/dl to >200 mg/dl (P = 0.001) while on therapy. Two patients suffered catheter-related infections requiring i.v. antibiotics. One patient developed reactivated genital herpes, genital warts, and a cutaneous candida infection requiring treatment. Serious vascular adverse events included a pulmonary embolus in week 6, an ischemic cerebrovascular event in week 4, and an upper extremity deep vein thrombosis from catheter placement followed by a gastrointestinal bleed (associated with bowel metastasis) in week 3, both in the same patient.

**Clinical Response.** Twenty-six of 31 patients were evaluable for response. Of the 5 nonevaluable patients, one expired after a pulmonary embolus, one expired after discharge from a hospitalization to treat dehydration, one did not continue treatment after hospitalization for pneumonia, one was removed from the study after a cerebrovascular accident, and the fifth one was deemed ineligible because of the discovery of a new central nervous system lesion. Response rates are as shown in Table 3. One patient, stage M1a, had a partial response after two cycles and went on to have stable disease for 16 additional cycles before progressive disease was observed. One patient, stage M1c (treated central nervous system disease) had stable disease after two cycles but progressed after the fourth cycle. Of note, among the patients with progressive disease, mixed responses were observed in 5 (16.7%), suggesting that a subset of their tumors responded to SU5416. Sites of response included skin (n = 2), skin and liver (1 patient), lung (1 patient), and perirectal region (1 patient); sites of progression included both known areas and new lesions. An example of substantial shrinkage of liver metastases in a patient with a mixed response is shown in Fig. 2. One mixed response patient had significant regression of all known lesions but was found to have central nervous system metastases during a work-up for headaches and was removed from study. The median time to progression was 50 days, and the median overall survival was 29 weeks.

**Dynamic Contrast MRI.** To control for intrapatient variability, only patients with a tumor in or near the liver were eligible for evaluation by dynamic contrast MRI as an assessment of the effects of SU5416 on tumor perfusion. Using this approach, normal liver could be used as a reference tissue at each time point. Five patients qualified for this analysis. A representative contrast uptake versus time curve is depicted in Fig. 3. A modest decrease in the ratio of peak contrast uptake in the tumor versus normal liver was observed at week 8 versus pretreatment in these patients (Fig. 4; P = 0.024).

**Plasma VEGF Levels.** Inasmuch as SU5416 is thought to inhibit signaling of VEGF via Flk-1, it was hypothesized that circulating VEGF levels might increase after treatment as less VEGF would be internalized after receptor binding. To this end, plasma VEGF levels were measured pretreatment and during week 8 of therapy by ELISA. Blood samples at both time points were successfully obtained from 13 patients. As shown in Fig. 5, VEGF levels were significantly higher after 8 weeks of SU5416 therapy compared with pretreatment (P = 0.008). There was no obvious correlation between pre- or posttreatment VEGF levels and either clinical response or adverse events. The plasma levels (expressed as pg/ml) in the 2 nonprogressing patients went from 137 to 138 and 14 to 81. In the 2 patients analyzed with a mixed response, the VEGF levels went from undetectable to 53 and 220 to 225. Only 1 of the 13 patients tested demonstrated a drop in VEGF levels (68 to 60); this patient had progressive disease.

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**Fig. 2** Example of tumor regression in a patient with a mixed response. A magnetic resonance image through the liver pretreatment (left panel) and after 8 weeks of SU5416 (right panel) in a patient experiencing a mixed clinical response.
DISCUSSION

The vascular nature of metastatic melanoma, the high expression of VEGF in this tumor, and the provocative preclinical data targeting VEGF in melanoma xenografts motivated this investigation of the Flk-1 tyrosine kinase inhibitor SU5416 in patients with advanced melanoma. The design of this trial included requiring documented progressive disease as an eligibility criterion to enable use of disease stabilization as a study end point. The rationale was that an antiangiogenic agent may be exclusively cytostatic and thus not cause objective tumor regression. In fact, stable disease was observed in only 1 patient on this trial, whereas 1 patient had an objective partial response lasting 18 months, and 5 additional patients had shrinkage of at least one tumor. Although the observation of mixed responses is complex and controversial, the shrinkage of several lesions in some of these patients suggests a true drug effect. These clinical observations suggest that future studies of antiangiogenic agents in melanoma should be pursued and could use standard eligibility and response criteria when considering trial design.

Although SU5416 was designed to target the Flk-1 tyrosine kinase, it theoretically could be affecting other kinases as well. Recent work has indicated that the activities of Flt-1 and Flt3 are also inhibited by SU5416 (22–24). An effect on Flt3 could explain the clinical response reported in a patient with relapsed AML (25). In addition, Flk-1 expression has been reported on melanoma cells themselves (26), suggesting the potential for a direct antitumor effect if this receptor can contribute to melanoma cell growth and/or survival in vivo. Although we did not investigate expression or phosphorylation of Flk-1 in this study, it would be of interest to explore these measures in future trials of similar agents.

![Fig. 3 Dynamic contrast magnetic resonance imaging tracing. The contrast uptake versus time curve in tumor (lower line) versus normal liver (upper line) in a representative patient pretreatment (A) and during week 8 of treatment with SU5416 (B).](image)

![Fig. 4 Dynamic contrast magnetic resonance imaging changes for 5 evaluable patients. The ratio of peak uptake in tumor versus normal liver was determined pretreatment (Pre) and during week 8 of therapy with SU5416, and values were compared using a paired t test. Each symbol represents one patient.](image)

![Fig. 5 Plasma vascular endothelial growth factor (VEGF) levels. The levels of circulating VEGF were measured in the plasma by ELISA. The mean was determined pretreatment (Pret) and during week 8 of therapy with SU5416, and values were compared using an unpaired t test.](image)
The results of the dynamic contrast MRI analysis on 5 patients in our current study support but do not prove an antiangiogenic mechanism of SU5416. The maximum contrast media uptake in the tumor is dependent on perfusion rate, capillary permeability, and volume of distribution. Of these, only perfusion rate and capillary permeability are indicators of angiogenesis. Methods of analyzing MRI data that can provide a more direct index of antiangiogenic activity are being developed. Although our current study compared the peak contrast uptake in the tumor to that in normal liver, recent work suggests that the area under the curve of the tumor perfusion alone may be a reliable parameter (27). Such an analysis would simplify application of this assay in future trials. Finally, all patients received corticosteroids along with anti-H1 and anti-H2 agents, which also could have affected tumor vascular perfusion. It is, therefore, not formally possible to discern a contribution of the premedications to the observed MRI effects, nor can we determine changes in tumor vascularity that could have resulted from the natural history of the disease.

Elevated plasma VEGF levels were observed during week 8 compared with pretreatment. Although this increase could theoretically have been a reflection of tumor growth, 1 patient with stable disease had an increase in serum VEGF levels, and the only patient with decreased posttreatment VEGF levels had disease progression. These observations suggest that the treatment inhibited VEGF utilization in at least some patients and that increased VEGF posttreatment was not always attributable to disease progression. As with the observations with MRI, it is also possible that the premedications contributed to this effect. A recent study suggested that high urinary levels of VEGF may correlate with clinical response (18). Thus, consideration should be given to measure urinary VEGF in future studies of VEGF-targeted therapies.

A significant difficulty with SU5416 was the biweekly i.v. administration schedule coupled with the requirement for steroid premedication due to the Cremophor vehicle. This amounted to four doses of dexamethasone weekly, which appeared to be sufficient to cause lymphopenia and hyperglycemia after 8 weeks. It is possible that the SU5416 itself contributed to these phenomena. Because melanoma is a tumor type that seems responsive by blunting a potential endogenous antitumor immune response. This specific agent also would not be logical to combine with immunological treatments.

SU5416 exhibited limited single-agent activity in our study. Preclinical data, however, suggests that combination studies of VEGF-targeting agents with other modalities such as chemotherapy, radiation therapy, or immunotherapy are worthy of consideration (29–31). However, the adverse consequences of chronic corticosteroid administration limit the use of SU5416 as the VEGF-targeted agent of choice.

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APPENDICES

Affiliate participants: John W. Kugler and James A. Knost, Oncology/Hematology Associates, Peoria, IL; David A. Taber and Rafat H. Ansari, Northern Indiana Cancer Research Consortium, South Bend, IN; and James L. Wade, Decatur Memorial Hospital, Decatur, IL.

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