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Dual VEGF/VEGFR inhibition in advanced solid malignancies

Clinical effects and pharmacodynamic biomarkers

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Our prior phase I study of the combination of vascular endothelial growth factor (VEGF) antibody, bevacizumab, and VEGF receptor (VEGFR) inhibitor, sunitinib, in advanced solid tumors identified an encouraging response evaluation. An expansion phase of this study was thus undertaken to obtain further safety data, response assessment and characterization of pharmacodynamic biomarkers in melanoma, renal, and adrenal carcinoma patients.

Patients with metastatic solid tumors received sunitinib (37.5 mg/d, 4 wk on/2 wk off) and bevacizumab (5 mg/kg intravenously every 2 wk). Responses were assessed every 2 cycles. Serum levels of angiogenic molecules were measured using ELISA assays.

Twenty-two patients were enrolled, including 11 melanoma, 5 renal cell carcinoma (RCC), 5 adrenal cancer, and 1 angiosarcoma. Grade 3 or higher adverse events were observed in 15 patients, including hypertension (41%), thrombocytopenia (23%), and fatigue (14%). Three RCC patients, and 1 melanoma patient developed thrombotic microangiopathy (TMA). Partial response (PR) occurred in 21% patients, including melanoma (2), adrenal (1), and renal (1) carcinomas. Overall, 6 patients demonstrated some reduction in their tumor burden. Serum VEGF and several other proangiogenic proteins declined over the first 4 wk of treatment whereas the putative VEGF-resistant protein, prokineticin-2, increased over 10-fold.

Occurrence of TMA related to dual VEGF/VEGFR inhibition can result from systemic or nephron specific injury even in non-renal malignancies. While the combination of sunitinib and bevacizumab was clinically efficacious in renal cell carcinoma and melanoma, the observance of microangiopathy, even in non-RCC patients, is a significant toxicity that precludes further clinical development.

Introduction

The vascular endothelial growth factor (VEGF) and VEGF receptors (VEGFRs) play a pivotal role in tumor growth and metastasis. Emerging data also suggest their involvement in cell differentiation, survival, motility, and tumor invasion.[1] Interest in therapeutic implications of anti-angiogenesis originated with the isolation of "tumor angiogenesis factor" and the discovery of its mitogenic effects on endothelium.[2,3] Vascular permeability factor was subsequently discovered[4,5] and later, identified as VEGF.[6,7] In 2003, recombinant monoclonal antibody against circulating VEGF, bevacizumab, demonstrated antiangiogenic clinical efficacy.[8] Subsequently, inhibitors of VEGFRs, small molecule tyrosine kinase inhibitors (TKI) were developed that markedly improved treatment of several malignancies. Sunitinib, a TKI inhibitor of VEGFRs 1, 2 and 3, additionally targets c-KIT, FMS-like tyrosine kinase 3, PDGF-α, PDGF-β, and RET signaling pathways.[9]

Lack of complete regression of tumor vasculature and development of resistance to targeted agents have necessitated quest for alternative anti-angiogenic strategies. These have included newer targeted inhibitors as well as concomitant targeting of VEGF ligands together with VEGF receptors. Sunitinib and bevacizumab inhibit angiogenesis at complementary sites; dual VEGF and VEGFR inhibition has the potential to achieve a more thorough inhibition of the VEGF signaling pathway, which could, in theory, improve anti-tumor efficacy.[10,11] The estimated half-lives of bevacizumab and sunitinib with its active metabolite, are 21 d (range of 11–50 d) and 1–3 d respectively. Angiogenic adaptation during combined treatment with bevacizumab and sunitinib occurring in the first 4 wk reflects the effect of combined VEGF and VEGFR inhibition. Between weeks 4 and 6, during the standard treatment break ("off-period") of sunitinib, rebound changes in the VEGF receptors and associated cytokines are expected, with continued suppression of ligands engaged directly by VEGF. Combined treatment with bevacizumab and sunitinib has previously been evaluated in phase I studies of patients with advanced renal cell carcinoma and other solid tumors.[11,12] In addition to metastatic renal cell carcinoma (mRCC), initial studies suggested that this combination might have activity in melanoma and adrenal cortical

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Table 1. Toxicity data: Grade ≥3 toxicities and toxicities of special interest

Grade	≥3	All grade	≥3	All grade
Toxicity	***n***	***n***	**%**	**%**
Fatigue	3	17	13.64	77.27
Hypertension	9	16	40.91	72.73
Thrombocytopenia	5	15	22.73	68.18
Nausea	1	13	4.55	59.09
Oral mucositis	2	12	9.09	54.55
Anemia	1	8	4.55	36.36
Lymphopenia	1	8	4.55	36.36
Diarrhea	1	7	4.55	31.82
Neutropenia	2	6	9.09	27.27
Palmar–plantar erythrodysesthesia syndrome	1	5	4.55	22.73
ALT elevation	1	4	4.55	18.18
Thrombotic microangiopathy	1	4	4.55	18.18
Pain	1	4	4.55	18.18
Hypophosphatemia	1	4	4.55	18.18
Haptoglobin reduction	0	4	0.00	18.18
Vomiting	1	3	4.55	13.64
Abdominal pain	1	2	4.55	9.09

carcinoma.[13] Also noted in these studies, however, was the occurrence of thrombotic microangiopathy (TMA), at least in RCC patients with prior nephrectomies. Taken together with other published reports of TMA in RCC patients,[12,14] the combination of sunitinib and bevacizumab may be associated with microangiopathy at both low as well as high doses in patients with mRCC.

We initiated an expansion of our phase I study of bevacizumab and sunitinib, primarily to obtain further safety data in non-RCC tumor types. Secondary goals were to assess objective tumor responses, and to characterize dynamics of angiogenic proteins that might offer insights into mechanisms of tumor response and resistance.

Results

Patient characteristics

Twenty-two patients were enrolled in the expansion cohort, including ten males and 12 females. The median age at enrollment was 58 y. Tumor types included: melanoma (11), adrenal cancer (5), RCC (5), and angiosarcoma (1). Prior systemic treatment included chemotherapy (41%) and immunotherapy (27%). All 5 RCC patients, 3 of the 5 ACC patients and 2 of the 5 melanoma patients had received no prior treatment. Common sites of metastatic disease included lymph nodes (64%), lung/pleura (45%), and liver (32%).

Exposure and safety

A median of 2 cycles per patient were completed (range, 0–8). Upon learning of the joint National Cancer Institute and the Case Comprehensive Cancer Center decision to stop further accrual of RCC patients due to risk of microangiopathy,[14] one RCC patient decided to withdraw from the study on day 12. One ACC patient was hospitalized during cycle 1 for severe abdominal pain related to underlying bulky tumor mass. She developed worsening respiratory failure (from suspected pneumonia and/or undocumented pulmonary embolism) that eventually resulted in death. A second ACC patient developed chest pain, ST elevations, and cardiogenic shock during cycle 1. An autopsy identified acute myocardial infarction involving the basal interventricular septum and left lateral ventricular wall, with normal coronary lumens. This cardiovascular toxicity was felt by the investigators to be at least possibly related to treatment.

Asymptomatic grade 4 thrombocytopenia occurred in two patients. Three RCC patients who had previously undergone nephrectomy, developed TMA, reported in part previously.[14] One melanoma patient (without nephrectomy) developed low haptoglobin (grade1), thrombocytopenia (grade 3), acute renal failure (grade 2), and schistocytes, consistent with a diagnosis of hemolytic uremic syndrome (a subtype of TMA). This occurred during cycle 2. Discontinuation of treatment led to reversal of TMA in all patients. With the fourth occurrence of TMA, particularly its occurrence in a non-RCC patient, a decision was made to close the trial prior to completion of planned enrollment.

Common all-grade toxicities included fatigue (77%), hypertension (73%), thrombocytopenia (68%), nausea (59%), and oral mucositis (55%) (**Table 1**). Less commonly, anorexia, dysgeusia, and leucopenia were noted. Adverse effects ≥grade 3 occurred in 15 patients (68%); these included hypertension (41%), thrombocytopenia (23%), and fatigue (14%). Seven patients developed hemorrhagic events, all grade 1 or 2.

Sunitinib was dose-reduced in three patients, and held in at least one cycle in 13 patients. The most common reason was hypertension. Other reasons included fatigue, bleeding, thrombocytopenia, mucositis, acute renal failure, neutropenia, diarrhea, and to facilitate wound healing. Bevacizumab was withheld in one or more cycle(s) in 11 patients. The most common reason for suspension was hypertension; another significant indication was TMA as described above. One patient decided to come off-study after completing cycle 1 due to adverse effects (fatigue), while another came off study after completing 6 cycles due to a combination of adverse effects (diarrhea, stomatitis) and progressive disease on staging.

Therapeutic activity

Tumor response was evaluable in the 19 patients who completed 6 wk or more of treatment. Partial responses (PR) resulted in 21% (4/19) patients including one melanoma and three RCC patients. The melanoma patient, who had been refractory to chemotherapy and immunotherapy, developed a durable response to therapy lasting 7 mo, with a progression free survival (PFS) of 10.2 mo. The duration of responses for the three RCC patients with PR varied from 3 to 36 mo. Stable disease (SD) occurred in 16% (3/19) patients. One ACC patient with SD had end of study scans performed at 6 wk when consent was withdrawn secondary to fatigue. This patient demonstrated stable disease for 6 mo

after this brief exposure to therapy without need for additional treatment. The other two patients with SD included a melanoma patient (SD lasting for 2 mo), an RCC patient, with SD for 21 mo. Progressive disease occurred in 63% (12/19) patients. Overall, 6 patients had measurable reduction in tumor burden, varying from 21% to 66%. **Figure 1** summarizes the best therapeutic responses. Overall, the median PFS was 2.4 mo, with a range from 0.4 to 10.2 mo.

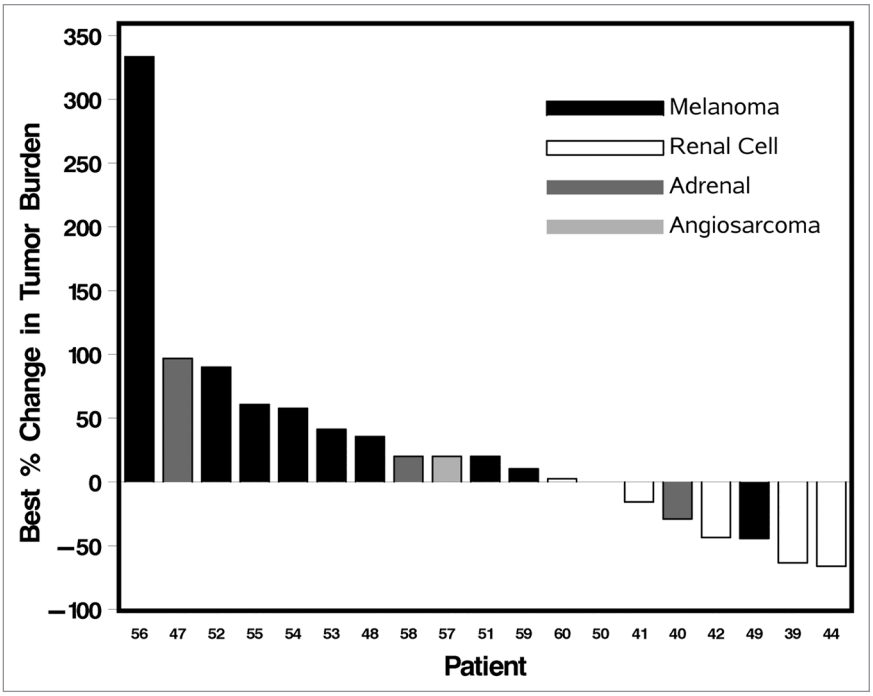

Figure 1. Waterfall plot of best responses. Patients with new lesions were empirically assigned a 20% increase in target tumor measurement for accurate reflection of best response.

Angiogenic biomarkers

Serum VEGF levels declined over the first 4 wk of treatment with this combination, but more markedly at the end of six weeks of bevacizumab ($P = 0.03$) (**Table 2**). CECs increased during the combined administration in the first 4 wk interval, from a median of 6.5/mL to 17.75/mL ($P = 0.03$), but returned to baseline when sunitinib was withdrawn. Between weeks 1 and 4, serum levels of the extracellular domain of the cytokine VEGF receptors (sVEGFR-2 and sVEGF-3) declined (both $P = 0.0002$); these changes were sustained during the two weeks without sunitinib administration (**Table 2**). Also consistent with dual inhibition of angiogenic drivers, were declines in sTie-2 ($P = 0.0002$), Ang-2 ($P = 0.0002$), endoglin ($P = 0.01$), and matrix metalloprotease 9 (MMP9) ($P = 0.01$). On the other hand, CXCL10 ($P = 0.01$) and vascular cell adhesion protein VCAM-1 ($P = 0.006$) increased during the first 4 wk.

The pro-angiogenic factor prokineticin-2 also increased >10× over the first 4 wk ($P = 0.01$), a change that was not sustained after the end of the phase of dual inhibition. Prokineticin-2 levels were undetectable at baseline in 3 melanoma patients. Two of these patients, who had progressive disease at 10 wk, had

Table 2. Markers of angiogenesis in patients treated with dual VEGF/VEGFR inhibition

	Median level baseline ($n = 13$)[a]	Median level week 4 ($n = 13$)[a]	Median level week 6 ($n = 9$)[a]	*P* value[b] baseline vs week 4	*P* value[b] baseline vs week 6	*P* value[b] week 4 vs week 6
CEC (cells/mL)	6.5	17.75[c]	6.12[d]	**0.03**	0.85	0.64
VEGF (pg/mL)	360.67	217.19	167.66	0.07	**0.03**	**0.004**
VEGFR1 (pg/mL)	109.32	149.33	117.35	1.0	0.30	0.50
VEGFR2 (pg/mL)	1856.1	1264	1393.1	**0.0002**	**0.004**	**0.03**
VEGFR3 (ng/mL)	45.04	15.38	23.66	**0.0002**	**0.004**	**0.004**
Endoglin (ng/mL)	3.69	3.23	3.34	**0.01**	**0.004**	0.82
TIE-2 (ng/mL)	20.77	15.48	17.42	**0.0002**	**0.004**	**0.03**
VCAM-1 (ng/mL)	576.6	932.18	932.18	**0.006**	**0.04**	0.73
Angiopoeitin-2 (pg/mL)	2841.4	1537.2	1843.8	**0.0002**	**0.004**	**0.04**
MMP-9 (ng/mL)	609.3	312.8	363.6	**0.01**	**0.03**	0.25
CXCL-10 (pg/mL)	124.2	347.41	154.51	**0.01**	0.20	**0.04**
Prokineticin-1 (ng/mL)	0.615	0.646	0.54	0.22	1.0	0.57
Prokineticin-2 (pg/mL)	0.35	3.95	0.83	**0.01**	0.11	0.43
S100A9 (ng/mL)	3.26	2.55	2.8	0.64	0.10	0.73

[a]Unless otherwise noted; [b]Wilcoxon signed rank test; [c]$n = 11$; [d]$n = 10$.

significant upregulation of prokineticin-2 (week 4 levels 3.95 ng/mL for both). A third melanoma patient, who had a partial response, expressed only a minor increase to 0.1 ng/mL that reverted to undetectable levels at week 6. Interestingly, this melanoma patient also had significantly lower CXCL-10 levels at all time-points in cycle 1, as well as higher baseline MMP-9 (nearly twice the group median).

Discussion

Angiostatic responses have been evaluated in RCC and melanoma in both pre-clinical and clinical studies. In addition to the rationale for study in RCC, vascularity has been correlated with clinical outcome and survival in melanoma.[15-17] Furthermore, because of their aggressiveness, murine melanomas were used in early studies of VEGF to define the role of angiogenesis in the metastatic cascade.[5,18-22] While the combination of sunitinib and bevacizumab was clinically efficacious in renal cell carcinoma and melanoma, the observance of microangiopathy, even in non-RCC patients, was a significant toxicity that led to early closure of this study and probably precludes further clinical development of this combination. TMA is characterized by development of occlusive microvascular thrombi, microangiopathic hemolytic anemia, consumptive thrombocytopenia, and organ ischemia.[23,24] TMA in RCC may be pathophysiologically linked to podocyte specific VEGF disruption in the glomerular microvasculature of the solitary kidney, since many of these patients have previously undergone nephrectomy.[25] However, the novel finding of TMA related to dual VEGF/VEGFR inhibition in a melanoma patient suggests that microangiopathy can result from either systemic or nephron specific endothelial injury even in patients with adequate glomerular reserve and in non-renal malignancies.

Clinical exploration of alternate angiogenic molecules to overcome resistance has been a growing focus of pharmacodynamic and translational studies of anti-antiangiogenics. Interest in the evaluation of angiogenic changes in VEGF and alternate pathways is 2-fold. While mechanistic roles of alternate angiogenic proteins might identify novel therapeutic targets, unraveling their correlation with response and resistance could lead to development of predictive biomarkers. Vascularity and vascular endothelial growth factor (VEGF) are adverse prognostic factors in melanoma.[15-17,26] Tissue analyses in RCC patients undergoing neo-adjuvant treatment with sunitinib identified suppression of VEGFR-1 and VEGFR-2 gene expression.[27] Ang-2 is a cytokine in the tumor microenvironment that binds to TIE-2, an endothelial cell receptor tyrosine kinase of the Tie family, and affects endothelial cell survival and proliferation.[28] MMPs are ligands for integrins expressed on the surface of endothelial cells (EC); they have an established role in EC migration and invasion, both of which are essential for vessel sprouting.[29,30] Another family of alternate angiogenic molecules is prokineticins. Prokineticin-1 and 2 promote tissue-specific angiogenesis and hematopoietic cell mobilization.[31,32] Elevated levels of VEGF, isoforms of VEGFR, Ang-2, Tie-2, and MMP9 have all been associated with outcomes in melanoma.[33-43]

The observed shifts in angiogenic proteins in this study offer insights into angiostatic responses. The most profound effect of dual VEGF/VEGFR inhibition was observed in prokineticin-2 that increased over 10-fold. That this upregulation was significantly higher during the phase of dual VEGF/VEGFR inhibition, suggests a mechanistic link via VEGF receptors. Prokineticin-2, which can be upregulated by G-CSF, has also been shown to mediate resistance to bevacizumab in murine models.[44-47] This functional change in these patients, while potentially of importance, will need further validation. Another interesting trend was the dynamics of the Ang-2 and Tie-2 pathway. Serum Ang-2 levels are higher in patients with metastatic melanoma when compared with early stage disease.[37,48] Antibodies targeting Ang-2, are currently being evaluated in advanced solid tumors in phase I and II studies.[49,50] Third, MMP-9 changes mirrored those of VEGF. VEGF/VEGFR2 interaction can downregulate MMP-9 expression at the transcriptional level, thereby inhibiting cellular migration.[51] Our results provide further evidence that MMP-9 is interlinked with the VEGF pathway. Overall, these results demonstrate significant trends in alternate angiogenic proteins that provide hypothesis generating data for future studies.

Circulating endothelial cells (CECs) are impacted by disruption of tumor vasculature, and play an important role in the tumor microenvironment. CECs have been shown to be elevated at baseline in cancer patients when compared with healthy controls.[52-55] In mRCC, CECs initially increase with treatment, and subsequently declined toward baseline.[56] In uveal melanoma patients, adjuvant treatment with interferon-α-2b resulted in increased CECs, most apparent after 8 wk of treatment.[57] Increase of CECs from baseline was observed in patients assessed during the period of dual VEGF/VEGFR inhibition. The mechanistic basis of CEC upregulation, possibly reflecting a disruption in tumor vasculature, is not completely understood. However it may be noteworthy that CD146, an endothelial biomarker used to isolate CECs, is a coreceptor for VEGFR-2 on endothelial cells.[58]

In summary, combined treatment with bevacizumab and sunitinib in patients with melanoma and renal cell carcinoma has clinical activity but limiting angiopathic toxicity precluding further clinical development. Angiostatic responses that were characterized identified significant downregulation of the pro-angiogenic proteins VEGF, sVEGFR2, sVEGFR3, endoglin, sTIE2, angiopoietin 2, and MMP-9 and upregulation of the proangiogenic prokineticin-2, sVCAM-1, and CXCL10. Further research will be required to elucidate the mechanistic roles of these and other non VEGF proteins in mediating resistance to antiangiogenic agents.

Patients and Methods

This study was approved by the Institutional Review Board of the Case Comprehensive Cancer Center and was registered at http://clinicaltrials.gov (NCT00357318). Patients aged 18 or older with histologically proven, metastatic/unresectable solid tumors not amenable to curative surgical or radiation therapy were enrolled in the expansion arm of our phase I study. Patients with

squamous cell histology or any histology in close proximity to a major blood vessel were excluded. Patients were enrolled if they had a performance status of ECOG 0 or 1, reported resolution of acute toxic effects of prior therapy, radiotherapy, or surgical procedure; and demonstrated adequate organ function as defined by aspartate/alanine transaminase ≤2.5× upper limit of normal (ULN), bilirubin ≤1.5× ULN, absolute neutrophil count (ANC) ≥1500/μL, platelets ≥100000/μL, hemoglobin ≥10.0 g/dL, calcium ≤12.0 mg/dL, creatinine ≤1.5× ULN, and urine protein creatinine (UPC) ratio as determined by urinalysis <0.5 (for UPC ratio >0.5, 24-h urine protein level should have been <1000 mg for patient enrollment).

Patients who had previously received bevacizumab or sunitinib were excluded from the study. Patients were also excluded if they had received prior systemic therapy or radiation therapy within 4 wk of starting treatment on protocol. Furthermore, patients with the following conditions were excluded: bleeding diathesis or coagulopathy, history of or known brain metastases, spinal cord compression, or carcinomatous meningitis, new evidence of brain or leptomeningeal disease on screening CT or MRI scan unless without progression on MRI or CT for 3 mo, ongoing cardiac dysrhythmias of NCI CTCAE grade ≥2, atrial fibrillation of any grade, prolongation of the QTc interval to >450 ms for males or >470 ms for females, history of serious ventricular arrhythmia (VT or VF ≥3 beats in a row), conditions classified as NYHA III or IV, patients on full-dose anticoagulants (however patients receiving low-dose anticoagulation therapy were eligible), hypertension uncontrolled by medications to <140/90 mmHg, history of abdominal fistula, gastrointestinal perforation, or intra-abdominal abscess within the previous 28 d, serious, non-healing wound, ulcer, or bone fracture, hypersensitivity of Chinese hamster ovary cell products or other recombinant human antibodies, human immunodeficiency virus (HIV), or acquired immunodeficiency syndrome (AIDS)-related illness, current treatment on another clinical trial, and pregnancy or breastfeeding, or any of the following within the preceding 12 mo: myocardial infarction, severe/unstable angina, severe peripheral vascular disease (claudication), or procedure on peripheral vasculature, coronary/peripheral artery bypass, graft, New York Heart Association (NYHA) grade II or greater congestive heart failure, cerebrovascular accident or transient ischemic attack, clinically significant bleeding, deep venous thrombosis, or pulmonary embolism.

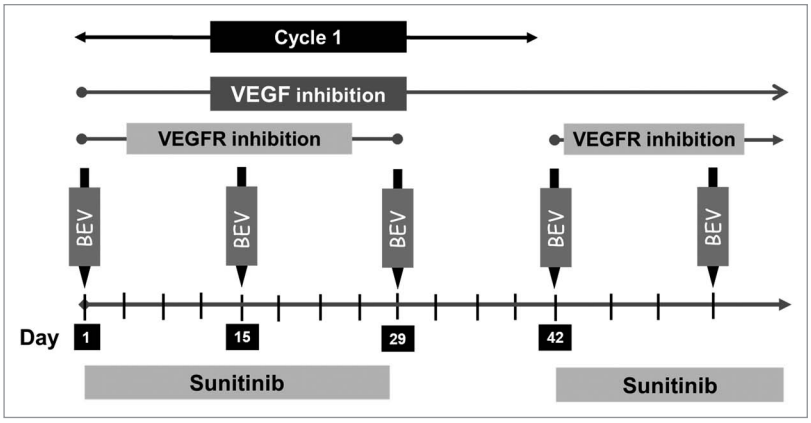

Figure 2. Study schema: Patients received sunitinib 37.5 mg PO daily from weeks 1–4 and bevacizumab (Bev) 5 mg/kg intravenously on days 1, 15, and 29 of each 6-wk cycle.

Study design and treatment

Patients received 37.5 mg sunitinib orally once a day from weeks 1–4 in addition to bevacizumab 5 mg/kg intravenously on days 1, 15, and 29 of each 6 wk (42 d) treatment cycle (**Fig. 2**). This dose schedule corresponded with level +1 of the original phase I trial. Doses were based on patient's actual body weight. Treatment was continued till patients developed any of the following: progressive disease per RECIST criteria, unacceptable adverse effects, intercurrent illness that precluded further administration of therapy, or patient decision to withdraw consent. Adverse events were graded according to the NCI Common Terminology Criteria for Adverse Events version 4.0. Dose limiting toxicities (DLTs) were defined as: any grade 4 toxicity (except lymphopenia or increased uric acid), any grade 3 cardiac event (except hypertension) or grade 3 venous thrombosis, hypertension unable to be controlled to <160/90 with oral medications within 4 wk, any grade arterial thromboembolic event, any grade 3 non-cardiac toxicity that failed to resolve to ≤ grade 1 within 6 wk (except proteinuria, lymphopenia, hypophosphatemia, and asymptomatic hyperamylasemia/hyperlipasemia) and/or proteinuria >3.5 g/24 h.

Dose modification

Sunitinib dose was reduced in patients experiencing non-dose limiting toxicities, based on individual patient tolerance. Sunitinib dose was delayed if the elevation of ALT/AST was greater than 5 times the ULN, or bilirubin was more than 3 times the ULN. Sunitinib could be re-administered when levels of ALT/AST and bilirubin declined to ≤5× and ≤3 ULN. There were no reductions in bevacizumab dose, rather the dose was held in case of adverse events, and was restarted at the same dose upon resolution on non-dose limiting toxicities.

Study assessment

Patients with measurable disease were assessed by RECIST criteria version 1.0 at baseline and day 28 of even numbered cycles.

Correlative studies

Circulating endothelial cells (CECs) and soluble angiogenic proteins were characterized in 10 melanoma patients and 3 additional patients (one each mRCC, angiosarcoma, and ACC) during cycle 1. Blood samples were collected from these 13 patients at baseline and week 4, and from 9 patients at week 6. Peripheral blood samples were drawn in one 10 mL CellSave tube, and one 10 mL serum tube. The serum samples were stored for batch analyses of regulatory circulating angiogenic proteins. Angiogenic proteins were quantified in serum samples at baseline, week 4, and week 6, using solid phase ELISA assays employing a quantitative sandwich immunoassay (all antibodies were obtained from R&D Systems except prokineticin 1, prokineticin 2, and S100A9 that were from Antibodies on Line). The

CellTracks® AutoPrep® System and the CellSpotter® Analyzer II System (Veridex, LLC) were used to enumerate circulating endothelial cells (CECs), using immunomagnetic separation.[57,59] Briefly, 4 mL of blood was used to enrich CD 146 positive cells using immunomagnetic separation. Subsequently, nuclear dye 4,6-diamidino-2-phenylindole (DAPI), and fluorochrome-conjugated monoclonal antibodies-phycoerythrin-conjugated CD105, and allophycocyanin-conjugated CD45, were added. Using image cytometry, CECs were defined as $CD146^+$, $DAPI^+$, $CD105^+$, and CD45 negative elements. Results were expressed as number of CECs per mL of blood.

Statistical design and data analysis

Statistical analyses were performed using SAS version 9.2 (SAS Institute Inc.). Correlative study data were analyzed using the Wilcoxon signed rank test and P values < 0.05 were considered significant. Data presented are medians. CECs were enumerated as cells per mL blood, while angiogenic proteins were quantified per mL of serum. Progression-free survival was summarized using the Kaplan–Meier method; and was measured from the start of treatment to the date of documented progression, or the date off-treatment for patients who discontinued therapy early for adverse events.

Disclosure of Potential Conflicts of Interest

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