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Alliance A091103 a phase II study of the angiopoietin 1 and 2 peptibody trebananib for the treatment of angiosarcoma

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Abstract

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Conflict of interest None.

Introduction—Angiosarcomas are rare malignant endothelial cell tumors which have up-regulation of the angiopoietin system [e.g., Tie2 and Angiopoietin 2 (Ang2)]. Trebananib is a novel agent targeting Angiopoietin 1 and Angiopoietin 2.

Methods—Trebananib 30 mg/kg was administered weekly until progressive disease or unacceptable toxicity. The primary endpoint was response rate by RECIST v1.1. Correlatives included: (1) baseline tumor expression of Ang2/Tie2 by immunohistochemistry, (2) serum levels of Ang1 and Ang2, (3) pre- and post-treatment phospho-receptor tyrosine kinase and (4) MYC/FLT-4 amplification status.

Results—Sixteen patients were enrolled [median age 68 years (24–91), 38 % male, median number of prior therapies 2.5 (1–7)]. No responses were observed in 12 evaluable patients. Estimated median and 12-week progression-free survival rate were 7 weeks (95 % 6–8) and 25 % (95 % CI 11–58 %), respectively. Median overall survival was 28 weeks (95 % CI 17–48). There were two (12.5 %) patients who experienced grade 3 adverse event and one (6.3 %) patient who experienced grade 4 adverse event that was considered at least possibly related to treatment.

Conclusions—Trebananib was well tolerated. Lack of response in the first stage of a Simon 2 stage design led to closure of this study. Prolonged PFS was observed in four pts, lasting 3.4–5.5 months.

Keywords

Angiosarcoma; Trebananib; Angiopoietin system

Introduction

Angiosarcomas (AS) are rare, aggressive, malignant endothelial cell tumors of vascular or lymphatic origin, which constitute 1–2 % of all soft tissue sarcomas (STS) [1]. The incidence of AS is equally distributed among both sexes but tends to occur more commonly in older patients [2–4]. Chemotherapy is typically reserved for unresectable disease with response rates ranging from 10 to 30 % with angiogenesis inhibitors and cytotoxic agents [5–9]. Given the poor outcome in this rare disease, there is a need to elucidate the pathogenesis of AS which can, ultimately, lead to more effective therapies.

The role of angiogenesis and associated angiogenic factors have led to many clinical trials exploring the role of vascular endothelial growth factor (VEGF) inhibition in AS. The VEGF inhibitors sorafenib and bevacizumab have shown responses in patients with AS, but the overall response rate still remains <20 % [7, 10]. VEGF regulates endothelial cell proliferation, vascular permeability, vasculogenesis and angiogenesis [11]. Cooperation of VEGF with angiopoietin (Ang) ligands is necessary in the development of tumor vasculature [11]. Interaction of Ang with its associated receptor tyrosine kinase (RTK), Tie2, is also critical for angiogenesis, blood vessel maturation and maintenance of the vascular endothelium [11].

It has been demonstrated that AS has up-regulation of other vascular-specific RTKs, including Tie1, Tie2 and Ang2 [12, 13]. The presence of elevated protein levels of both Tie2 and Ang2 have been demonstrated in advanced human AS specimens [12, 13].

Trebananib is a novel peptide-Fc fusion protein or peptibody [14]. Trebananib sequesters Ang1 and Ang2, thereby preventing their interaction with Tie2 and inhibiting tumor endothelial cell proliferation and tumor growth. In preclinical studies, dual inhibition of Ang1 and Ang2 was shown to be superior to selective Ang2 inhibition to suppress angiogenesis in some postnatal settings [15]. Trebananib has been shown to inhibit the growth of multiple tumor xenografts in nude mice [14]. The antitumor effect was associated with a reduction of endothelial cell proliferation and significant increases in tumor cell apoptosis and necrotic tumor fraction; there was no increase in endothelial cell apoptosis suggesting that trebananib prevents tumor growth via inhibition of angiogenesis [14]. In a phase I study of trebananib in patients with solid tumors, 29 patients were evaluable for tumor response. Four patients had stable disease (SD) for >16 weeks: one each with soft tissue sarcoma, thyroid cancer, pseudomyxoma and submandibular adenocarcinoma. Trebananib was well tolerated with the most common toxicities of fatigue, peripheral edema, insomnia, upper abdominal pain, back pain and nausea. Trebananib was found to be safe as a monotherapy at intravenous doses up to 30 mg/kg weekly in patients with advanced solid tumors.

The Ang system is relevant in the development and growth of angiosarcoma. We hypothesized that inhibition of Ang 2 with trebananib would be an effective therapy in patients with angiosarcoma.

Patients and methods

Study design

This is a National Cancer Institute (NCI)/Cancer Therapy Evaluation Program (CTEP) sponsored, open-label, multicenter phase II study using trebananib, in patients with AS who have metastatic/unresectable disease conducted through the Alliance for Clinical Trials in Oncology (Alliance A091103). Trebananib was provided by NCI/CTEP in collaboration with AMGEN pharmaceuticals.

Patients

Patients having histologically confirmed angiosarcoma that was metastatic or unresectable were eligible. Other inclusion criteria include adequate performance status (ECOG Eastern Cooperative Oncology Group <2) and organ function, measurable disease by RECIST 1.1 and ability to provide informed consent. Patients could not have received >5 prior chemotherapy regimens. The protocol was reviewed and approved by the institutional review boards at all participating centers. Patients provided informed consent prior to enrollment.

Study procedures

Patients were treated with trebananib 30 mg/kg weekly. Each cycle consisted of 28 days and was repeated until the patient developed progressive disease or unacceptable toxicity. Adverse events were monitored every cycle and using the NCI Common Toxicity Criteria for Adverse Events version 4.0 (CTCAE v4.0). A pre-defined data safety monitoring board

toxicity stopping rule was used for monitoring. Pre- and post-treatment biopsies were performed in up to 10 patients treated at MSKCC.

Disease assessments occurred every other cycle (i.e., 8 weeks), beginning cycle 2. Response and progression were evaluated in this study using the new international criteria proposed by the revised response evaluation criteria in solid tumors (RECIST) guidelines (version 1.1) [1]. Patients were to be re-evaluated for disease status 8 weeks after achieving a CR or PR to confirm the assessment. Similarly, an SD was re-assessed at a minimum interval of 6 weeks. Patients with global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time, and not related to study treatment or other medical conditions, were considered to have PD due to symptomatic deterioration. For the primary endpoint of the study, a confirmed tumor response was defined to be either a CR or PR on two consecutive evaluations at least 4 weeks apart.

Duration of response was calculated as the time between first response and disease progression. Time to progression was calculated as the time between registration and the date of progression. Patients without progressive disease or lost to follow-up were censored at their most recent disease assessment. Progression-free survival (PFS) was calculated as the time between registration and the earlier of disease progression or death. Overall survival (OS) was calculated as the time between registration and death. Patients lost to follow-up were censored at their most recent date last known alive. All patients were to be followed until the earliest of either death or 18 months post-registration.

Correlative studies

Pre-treatment archival tumor tissue was evaluated specifically for baseline expression of Ang2 and Tie2 by immunohistochemistry (IHC.) Peripheral blood was collected at baseline, during treatment and after progression and evaluated by ELISA assay in order to assess serum protein levels. Amplification status of MYC and FLT4 was performed on archival tumor tissue by fluorescence in situ hybridization (FISH.) Pre- and post-treatment biopsies were performed in consenting patients treated at Memorial Sloan Kettering Cancer Center between week 4 and week 5 dose of trebananib. Baseline phospho-RTK status (including but not limited to Tie2, VEGFR-2, PI3K, MAPK) was evaluated in tumor tissue.

IHC for Ang2/Tie2 expression

The Ang2 (Santa Cruz Biotechnology, Santa Cruz, CA) and Tie2 (R&D Systems, Minneapolis, MN) antibodies have been validated in the Antonescu laboratory and against positive and negative controls. For these IHC studies, tissue was stained for Ang2 and Tie2. Ang2 and Tie2 quantification was assessed and scored in a 0–3+ scoring system. 2+ strong staining was to be indicated by 50 % staining of cancer cells, 1+ for weak or strong staining in 50 % of the cells, and >5 % staining was scored as 0. These analyses were conducted in the laboratory of Cristina Antonescu.

Quantification of serum angiopoietins (Ang1 and Ang2)

Serum blood samples were collected pre-treatment and at weeks 3,5,9 and off-treatment and analyzed in batch for circulating levels of Ang 1 and Ang2 utilizing the Human Quantikine

ELISA Kits (R&D Systems, Minneapolis, Minn, Catalog # DANG10 and DANG20, respectively). Protocol followed manufactures instructions. Serum samples were taken prior to initiation of treatment. Blood was drawn into serum tubes (10 mL) and centrifuged at 2,200g/min for 10 min. Serum was to be harvested, aliquoted and stored at 70 °C until use. Enzyme linked immunosorbent assays were used to measure the levels of Ang1 and Ang2 according to manufacturer's instructions. Serum samples of patients with tumor were diluted for Ang1 and Ang2 determinations with assay buffer 50- and 10-fold, respectively.

Assessment of baseline growth factor expression and phosphorylation status in pre- and post-treatment samples

Lysates were produced from frozen biopsy samples obtained pre-treatment and at the end and evaluated using the human phospho-RTK Array Kit from R&D Systems. This platform allows analysis of the phosphorylation status of 42 RTKs, including the VEGFR (1–3) and TIE (1–2) families, as well as several others. Kinases were captured by antibodies spotted in duplicate on a nitrocellulose membrane, and their phosphorylation was then detected using an HRP-conjugated pan-phosphotyrosine antibody followed by chemiluminescence detection. The system offers higher throughput and better sensitivity than IP-Westerns. Results of RTK analysis were confirmed by immunoblot. For the Western blots, lysates were prepared by cutting the snap-frozen tumor tissue into a small piece and then grinding it in RIPA lysis buffer using sample grinding kit (GE Health-care). Protein concentrations were measured using Bio-Rad protein assay dye (Bio-Rad) and equal amounts of protein (20 or 30 µg) were loaded on 4–12 % gradient gels (Invitrogen) and transferred to PVDF membrane (Immobilon, Millipore) or nitrocellulose membrane (0.45 µm, Thermo Scientific) for protein detection. After blocking with 5 % milk, membranes were probed with primary antibodies overnight. Bound antibodies were detected with horseradish peroxidase secondary antibodies (GE Healthcare) and visualized by enhanced chemiluminescence reagent (GE Healthcare).

Amplification of MYC

FISH on interphase nuclei from paraffin-embedded 4-µm sections was performed applying custom probes using bacterial artificial chromosomes (BACs), covering *MYC* (RP11-440N18), *TPD52* (RP11-668P13; RP11-880M10.) BAC clones were previously chosen according to USCS genome browser (<http://genome.uscs.edu>). The BAC clones were obtained from BACPAC sources of Children's Hospital of Oakland Research Institute (Oakland, CA) (<http://bacpac.chori.org>). DNA from individual BACs was isolated according to the manufacturer's instructions, labeled with different fluorochromes in a nick translation reaction, denatured and hybridized to pre-treated slides. Slides were incubated, washed and mounted with DAPI in an antifade solution. Two hundred successive nuclei were examined using a Zeiss fluorescence microscope (Zeiss Axioplan, Oberkochen, Germany), controlled by Isis 5 software (Metasystems). The genomic location of each BACs set was verified by hybridizing them to normal metaphase chromosomes.

Statistical methods

The primary endpoint for this trial was the proportion of confirmed tumor responses estimated by the number confirmed responses divided by the number of patients evaluable for response. All eligible patients who signed a consent form had begun treatment and had at least one post-baseline assessment were considered evaluable for response. Based on low response rates in prior advanced STS studies, a response rate of at most 5 % was the threshold used for clinical inactivity (H_0) in regards to confirmed tumor response rate, whereas an observed confirmed response rate of at least 20 % was considered promising (H_a) in this population. One or more confirmed responses within the initial 12 patients would allow expanded enrollment to 37 total patients. Four out of 37 patients with confirmed tumor responses was considered evidence that this treatment could be recommended for further testing in subsequent studies in this patient population. This Simon [16] design yielded 90 % power to detect a true confirmed response rate of at least 20 %, at 0.05 level of significance [16].

Patients determined ineligible after having started treatment were included in the assessment of toxicity. Adverse events are reported as a maximum severity per patient and type, across all cycles of treatment. All attributions collected for adverse events were reported unless otherwise noted. Summary statistics, frequency tables and categorical data analysis were used to describe and compare the distributions of toxicity and correlatives. The Kaplan–Meier method was used to estimate distributions of time to event endpoints [17]. The Log rank test was used to compare the distributions of time to event endpoints based on the classification of patients by tumor or serum expression levels. All analyses were conducted using SAS version 9.0. Unless otherwise noted, all statistical tests were 2-sided and p values >0.05 were considered statistically significant.

Results

Patient characteristics

From July 2012 to January 2014, a total of 16 patients were accrued to this study at a rate of 0.8 patients per month. Enrollment went beyond 12 patients because four patients were inevaluable for the primary endpoint of response rate. These patients were enrolled at Washington University School of Medicine (9), Memorial Sloan Kettering Cancer Center (4), Mayo Clinic-Rochester (1), Medstar Washington Hospital Center (1) and Carle Cancer Center (1). There were ten (62 %) female patients ranging in age from 24 to 91 years (median 68.5, range 24–91). Thirteen (84.2 %) patients were Caucasian, two (12.5 %) patients were Black or African–American and one (6.2 %) patient was Asian. The median number of prior therapy types was 2 (range 1–7), which included single-agent chemotherapy, dual-agent chemotherapy, surgery, radiation therapy and immunotherapy. The median number of single-agent and dual-agent chemotherapy regimens was 2 (range 1–5) and 1 (range 1–1), respectively. Seven (43.7 %) patients had prior radiation therapy. Overall, seven (44 %) patients had multiple metastatic sites; most common sites were the lungs, bones and lymph nodes. More common locations for primary tumors included (patients): head (3), scalp (2) and breast (3) (Table 1).

Patient outcome and efficacy

No confirmed responses were observed and accrual was closed, per the statistical design. The study was allowed to remain open between stages and to await response data within the initial 12 patients due to slow accrual, low toxicity, and to over accrue a few cases to replace patients deemed ineligible. There were four (25 %) patients classified as having achieved disease control (i.e., responding or stable for at least 3.4 months). Thirteen (81 %) patients have progressed. Commonly occurring sites of progression (patients) included pulmonary (6), bone (6), liver (5) and abdomen (2).

The estimates of median time to progression and PFS are 1.61 months (95 % CI 0.92–1.74) and 1.63 months (95 % CI 1.41–1.74), respectively. Nine patients have died with a median follow-up for surviving patients of 4.63 months (range 0.92–12.61). The estimated median survival was 6.44 months (range 3.97–11.10). The survival rate at 6 months was 54 % (95 % CI 32.6–90.3 %) (Table 2; Fig. 1).

Drug administration

Sixteen patients received a total of 40 cycles of treatment (median 2 cycles, range 1–6). There were four patients who did not complete the first dose of cycle 1. The percent of targeted dose administered was calculated as the total dose administered to the patient, divided by the total dose the patient was expected to receive per protocol. Overall, the median percent of targeted dose of trebananib was 100 % (range 50–100). The treatment was reduced in two patients and omitted 10 times in eight patients. Reasons for discontinuing treatment include: disease progression 13 (81.2 %), adverse events 2 (12.5 %) and death on study 1 (6.2 %) (Table 3).

Tolerability

All 16 patients are evaluable for adverse events. Overall, two (12.5 %) patients experienced a grade 3 adverse event and one (6.3 %) patient experienced a grade 4 adverse event that were considered at least possibly related to treatment. The grade 4 adverse event was thromboembolic and the grade 3 adverse events include anemia, thromboembolic and syncope.

Two (12.5 %) patients died during treatment of events considered unrelated to study treatment. A 79-year-old male received full doses of trebananib and then was reduced to 75 % of the targeted dose. The patient was hospitalized during cycles 2 (pleural effusion) and 5. On Day 22 of cycle 5, the patient collapsed and was rushed to the emergency room and placed into intensive care. This patient expired the next day due to a “pulseless cardiac arrest.” A 27-year-old female patient received only two doses of trebananib during cycle 1 after experiencing grade 3 pneumothorax, pulmonary edema, hypercalcemia, hyperkalemia and hyponatremia. This patient was reported as having disease progression, was hospitalized due to respiratory failure and died within 16 days of initiating treatment.

Correlative studies

Ang2/Tie expression, MYC status—This analysis included 14 patients who provided consent for sample collection and studies. At the time of this report, results were available

for immunohistochemical studies using archival tumor tissue. The frequencies of C-MYC/FLT4 amplification and Ang2/Tie2 expression levels were evaluated by disease control status (Table 4). Patients having longer disease control appeared more likely to have tumors exhibiting Ang2 expression (67 vs 38 %; $p = 0.55$). Given that the study terminated early due to lack of efficacy, sample size limits the ability to obtain meaningful statistical results.

Serum Ang levels—This analysis included six patients who provided consent for blood collection. At baseline, Ang1 levels were tenfold higher than Ang2 levels. Ang1 levels ranged from 6,897 to 86,470 pg/ml. The mean Ang1 level was 46,973.8 pg/ml. Ang2 levels ranged from 900.6 to 9,786.6 pg/ml. The mean Ang2 level was 6,905 ng/ml. After one dose of trebananib, serum Ang1 levels decreased in four of six patients (Fig. 2a). After one dose of trebananib, serum Ang2 levels increased in five of six patients (Fig. 2b). All patients appeared to have higher Ang1 and Ang2 levels at progression compared with baseline values. Given the small number of patients, a statistical analysis could not be performed.

Western blot—Three patients treated at MSKCC had pre- and post-treatment biopsies. After one cycle of treatment, all three patient's tumors had baseline expression of Tie2, PI3K, p-MEK and p-AMPK. For patient #1, there was also baseline expression of VEGFR2. All three patients showed a decrease in Tie2 expression with therapy. The post-treatment blot for patient #1 demonstrated a complete loss of protein expression of multiple downstream signaling proteins including PI3K, p-MEK and p-AMPK. For patient #2, there was no decrease in protein expression of any of these signaling events. For patient #3, there was loss of p-MEK and PI3K expression; this does not appear to be due to deficient protein loading as there appears to be adequate tubulin expression. Furthermore, there was strong expression of total MEK. This would suggest a direct effect on these downstream kinases. Each of these patients was on study for approximately 2 cycles. Although patient #3 demonstrated suppression of PI3K and MEK, this did not translate to clinical benefit (Fig. 3).

Discussion

Angiosarcoma is an endothelial cell tumor and although its pathogenesis is not clearly understood, the role of angiogenesis and associated angiogenic factors have lead to many clinical trials exploring the role of VEGF inhibition [5, 7, 9, 18, 19]. VEGF may play a crucial role in the development of AS since it regulates endothelial cell proliferation, vascular permeability, vasculogenesis and angiogenesis [20]. Cooperation of VEGF with Ang ligands is necessary in the development of tumor vasculature [11]. Further, interaction of Ang with its associated RTK, Tie2, is also critical for angiogenesis, blood vessel maturation and maintenance of the vascular endothelium [11]. Therefore, the Ang system may be equally as important in the pathogenesis of angiosarcoma.

We hypothesized that the Ang system may be a relevant target in AS and conducted a phase II study of trebananib, an inhibitor of Ang1 and Ang2, in the treatment of patients with unresectable or metastatic angiosarcoma. Since four patients were not evaluable for the primary endpoint of tumor response, enrollment went beyond the initially planned 12

patients in the first part of the Simon 2 stage design. Sixteen patients were treated on the study, with a majority being women (63 %). In this cohort of patients, six of 16 (38 %) had visceral primary sites of disease, including two cardiac primaries which typically behave more aggressively [21]. Although 16 patients were enrolled on the study, only 12 were evaluable for assessing tumor response. There were four patients who did not complete the first dose of drug administration during cycle 1 due to rapid disease progression. This highlights the aggressive nature of this disease as well as the advanced disease status of our enrolled patients.

There were no confirmed tumor responses observed and accrual was closed, per the statistical design. Of note, one patient had an 18 % decrease in tumor size. There were four (25 %) patients demonstrated disease control for at least 3.5 months. The estimates of median time to progression and PFS were 1.63 months (95 % CI 1.41–1.74) and 1.6 months (95 % CI 0.92–1.74), respectively. Nine patients have died with a median follow-up for surviving patients of 4.63 months (range 0.92–12.61). The estimated median survival was 6.44 months (range 3.97–11.10.). Patients with AS have a 5-year OS of approximately 35 % [2, 3, 21]. In our study, the 1-year survival was only about 13 %. This further supports the advanced patient population that was enrolled and lack of benefit provided by the study drug.

Trebananib was well tolerated with minimal toxicities demonstrated. Overall, two (12.5 %) patients experienced grade 3 adverse event and one (6.3 %) patient experienced grade 4 adverse event that was considered at least possibly related to treatment. The grade 4 adverse event was thromboembolic, and the grade 3 adverse events include anemia, thromboembolic and syncope. Two patients died on study, unrelated to treatment.

Our correlative studies yielded some interesting observations. We sought to evaluate baseline Ang2 and Tie2 IHC expression. The Tie2 receptors along with the Tie1 receptor belong to a larger family of RTKs [22]. Tie2 receptor expression is limited to endothelial cells [22, 23]. Therefore, it was not surprising that all of nine tested specimens expressed Tie2 since angiosarcoma is thought to be a tumor of endothelial cell origin. We noted Ang2 expression in five of 11 tested samples. In normal adult tissue, Ang2 can be found in sites of active vascular remodeling such as the ovary, placenta and uterus. Increased Ang2 expression is found in the vasculature of many malignant tumors and has also been correlated with worse histological grade, more advanced tumors stage and adverse prognosis [24, 25]. While we were unable to determine the prognostic significance of Ang2 expression, it appeared as though those patients with baseline Ang2 expression by IHC remained on study for longer compared to those that lacked expression. Future studies with this agent in angiosarcoma should consider selecting patients by baseline Ang2 IHC expression.

Serum blood samples of Ang1 and Ang2 were evaluated in six patients. In ovarian carcinoma, Ang1 and Ang2 levels were quantified from patient's serum samples and correlated with patient outcome [26]. It was higher Ang2 levels that correlated with more advanced tumor stage and was a predictor of poor overall and recurrence free survival, (p 0.042, p 0.043 and p 0.033), respectively [26]. In that analysis, Ang1 levels were typically

tenfold higher than Ang2 levels. In our analysis of six patients, we observed interesting trends. Ang1 levels were much higher than Ang2 levels. Patients appeared to have a decrease in Ang1 levels after the first dose of trebananib. Ang1 levels began to rise at the time of progression. Ang2 levels appeared to continue to rise, despite therapy with trebananib. Levels at the time of progression were greater than at baseline for both Ang1 and Ang2. Unfortunately, the limited amount of samples precluded a statistical analysis. These results suggest that trebananib may have an immediate biologic effect on Ang1 levels and Tie1 signaling, but that this effect is transient and reversible within the first several weeks of therapy. This in fact could suggest a mechanism of drug resistance that sets in even before the first imaging studies. It is unclear why Ang2 levels did not decrease after treatment since trebananib is supposed to bind both Ang1 and Ang2 with equal potency [14]. As the first plasma samples were not drawn until week #2, it is possible that we missed the decrease in Ang2 levels and these in fact represent increasing levels after an initial decline. It is also known that Ang2 expression can be regulated by other factors in the tumor microenvironment. Specifically, in vivo data have demonstrated that VEGF can increase Ang2 expression [27]. Perhaps the persistent increase in Ang2 is driven by VEGF and Ang blockade alone is not sufficient to down-regulate its expression. This requires further prospective evaluation but may support combination strategies of dual VEGF and Ang pathway blockade.

Ang2 stimulation of the Tie2 receptor can activate the mitogen-activated protein (MAP) kinase, phosphatidylinositol-3 kinase (PI3K) and protein kinase B (Akt) pathways [24]. We have previously demonstrated that a subset of patients with AS have mutations in the VEGF pathway and can potentially have upregulation of phospho-VEGFR-2 as well. Therefore, we analyzed phospho-RTK status of Tie2, VEGFR-2, PI3K, MEK and MAPK and correlated the presence or absence of each to response to therapy for three patients. Unfortunately, there was insufficient tissue to conduct the RTK assays. However, in our Western blots, we demonstrated that all three patients had baseline expression of Tie2, PI3 K, p-MEK, p-AMPK. There was one patient who had evidence of inhibition of p-MEK and p-PI3K and another with complete loss of all signaling pathways. This did not translate to clinical benefit. Interestingly, these biopsies were obtained at the time that Ang1 levels were again increasing. This could suggest that despite the administration of drug on a weekly schedule, the rising Ang1 levels prevents the ability to sustain inhibition of downstream signaling pathways. It also suggests that blocking the Ang pathway alone may not suffice and that alternate pathways are involved in promoting tumor growth in this disease.

As a result of increased Ang2 expression, blood vessels become more responsive to pro-angiogenic cytokines such as VEGF [28–31]. Therefore, VEGF may play crucial role in the pro-angiogenic effects of Ang2. Tie2 inhibition can also promote vascular regression in the absence of VEGF signaling. Therefore, consideration of dual blockade of VEGF and Ang pathways may lead to greater therapeutic benefit in this disease.

Conclusion

This phase II study did not meet the requirement of obtaining at least a partial response in one patient, leading to early closure. Trebananib appeared to be well tolerated, and there

may be a role for this agent in selected angiosarcoma patients whose tumor express Ang2. In addition, given the complexity of the Ang2/Tie2 pathway, consideration of blockade of the VEGF pathway may be warranted. Conducting in vivo analyses to identify the appropriate combination agent is necessary to plan a future study. Further, one must also consider appropriate endpoints in sarcoma studies. Pazopanib, a RTK inhibitor, was recently approved for sarcoma patients with a progression-free survival of about 4 months and an overall response rate of <5 % [32]. Determining that lack of response in a cohort of 12 patients may not be consistent with determining lack of efficacy. Nonetheless, we have demonstrated that conducting clinical studies in rare cancers with incidences of >200 patients per year in the USA is feasible through the Alliance Clinical Trials in Oncology National Clinical Trials Network.

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Abbreviations

PI3 kinase	Phosphatidylinositol-4,5-bisphosphate 3-kinase
p.MEK1	Phosphorylated mitogen-activated protein kinase/extracellular-signal-regulated kinase
T. MEK	Total mitogen-activated protein kinase/extra-cellular-signal-regulated kinase
T.AMPK	Total 5' adenosine monophosphate-activated protein kinase
VEGFR2	Vascular endothelial growth factor receptor 2

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Fig. 1.
Kaplan–Meier *curve* of progression-free survival (*red*) and overall survival (*blue*) for all patients treated on study ($n = 16$, median progression-free survival 1.63 months, median overall survival 6.44 months)

Fig. 2.

a Serum Angiotensin 1 level, pg/ml ($n = 6$). Each patient is indicated by a different *color*. Each *dot* represents a blood draw for serum Angiotensin level. Patients 1, 4, 5 and 6 appeared to have a decrease in their serum Angiotensin 1 levels after dose 1 of trebananib. **b** Serum Angiotensin 2 level, pg/ml ($n = 6$). Each patient is indicated by a different *color*. Each *dot* represents a blood draw for serum Angiotensin level. All patients except patient five had an increase in their Angiotensin 2 levels after 1 dose of trebananib

Fig. 3.

Western blot of pre- and post-treatment biopsies treated at Memorial Sloan Kettering Cancer Center ($n = 3$ patients.). Each patient is indicated by the number on top. The pre-treatment is indicated by the -, the post-treatment by the +. Each of these patients was on study for two cycles. For patient #1, there was loss of protein expression of downstream signaling pathways leading to loss of pathway activation of Tie2, PI3K, p-MEK and p-AMPK in the post-treatment specimen (*red box*). For patient #2, there was no inhibition or protein loss (*green box*). For patient #3, there was inhibition of p-MEK and PI3K; this is not due to protein loss as the protein band is still there. This indicates pathway inhibition (*blue box*)

Table 1Patient characteristics ($n = 16$)

Characteristic	Frequency/estimate
Age, median (range)	68.5 (24.0–91.0)
Gender	
Female	10 (62.50 %)
Male	6 (37.50 %)
ECOG performance score	
0	10 (62.50 %)
1	6 (37.50 %)
Previous cancer ^a	
Yes	6 (37.5 %)
No	10 (62.5 %)
Primary tumor site	
Head (skull, face)	3 (18.8 %)
Scalp	2 (12.5 %)
Radiation-associated breast	1 (6.3 %)
Extremity, Stewart Treves	1 (6.3 %)
Breast	3 (18.8 %)
Cardiac	2 (12.6 %)
Other (spleen, abdomen, peri-rectal, unknown primary)	4 (25 %)
Prior therapies median (range)	2 (1–7)
Chemotherapy, single agent	2 (1–5)
Chemotherapy, multiple agent	1 (1–2)
Surgery	1 (1–3)
Radiation	1 (1–2)
Immunotherapy	1 (1–1)
Hormonal Therapy	1 (1–1)
Prior radiation	
Yes	7 (43.7 %)
No	9 (56.2 %)

^aPrior cancers included breast and ductal carcinoma in situ

Table 2

Treatment outcomes

	Frequency/estimate
Time to progression	
<i>N</i> (events)	16 (13)
Median	1.61 (95 % CI 0.92–1.74)
3 Month event rate	26.7 % (95 % CI 11.5–61.7 %)
Progression-free survival	
<i>N</i> (events)	16 (15)
Median	1.63 (95 % CI 1.41–1.74)
3 Month, event rate	25 % (95 % CI 10.7–58.4 %)
Overall survival	
<i>N</i> (events)	16 (9)
Median	6.44 (95 % CI 3.97–11.10)
3 Month, event rate	87 % (95 % CI 71.8–100 %)
6 Month, event rate	54 % (95 % CI 32.6–90.3 %)
9 Month, event rate	43 % (95 % CI 22.2–85 %)
12 Month, event rate	14.5 % (95 % CI 2.6–82 %)

CI confidence interval

Table 3

Treatment administration

Number of cycles administered	40
Median number of cycles per patient (range)	1 (1–6)
Number of patients with dose adjustments/omissions/modifications	8 (50 %)
Total doses omitted	10
Reasons for omission	
Hematological adverse event	1 (6.5 %)
Non-hematological adverse event	
Pulmonary	4 (25 %)
Urinary tract infection	1 (6.25 %)
Other ^a	4 (25 %)

^aOther non-hematologic reasons for omissions include: Other, not per protocol (scheduling error) 1 (6.25 %), general disorders and administration site conditions 1 (6.25 %), investigations 1 (6.25 %) and disease progression 1 (6.25 %)

Table 4

Ang/Tie 2 expression, MYC and FLT 4 status

	Disease progression <3.5 months (<i>n</i> = 10)	Disease progression 3.5 months (<i>n</i> = 4)	Total (<i>n</i> = 14)	<i>P</i> value
Ang2 expression status				
Positive	3 (37.5 %)	2 (66.7 %)	5 (45.5 %)	0.55
Negative	5 (62.5 %)	1 (33.3 %)	6 (54.5 %)	
Missing	2	1	3	
Tie2 expression status				
Positive	6 (100 %)	3 (100 %)	9 (100 %)	1.00
Negative	–	–	–	
Missing	4	1	5	
C-MYC status				
Amplified	2 (25 %)	0 (0 %)	2 (18.2 %)	1.00
Not amplified	6 (75 %)	3 (100 %)	9 (81.8 %)	
Missing	2	1	3	
FLT-4 status				
Amplified	1 (12.5 %)	0 (0 %)	4	1.00
Not amplified	7 (87.5 %)	2 (100 %)	1 (10 %)	
Missing	2	2	9 (90 %)	

Ang2 angiopoietin 2, *FLT-4* Fms-related tyrosine kinase 4