Published in final edited form as:
*Int J Radiat Oncol Biol Phys*. 2011 November 15; 81(4): 1081–1090. doi:10.1016/j.ijrobp.2010.07.024.

# Phase II Study of Neoadjuvant Bevacizumab and Radiation Therapy for Resectable Soft Tissue Sarcomas

**Sam S. Yoon, M.D.**[1], **Dan G. Duda, Ph.D.**[2,9], **Daniel L. Karl, B.S.**[1,9], **Tae-Min Kim, Ph.D.**[7,9], **Avinash R. Kambadakone, M.D.**[3], **Yen-Lin Chen, M.D.**[2], **Courtney Rothrock, B.S.**[1], **Andrew E. Rosenberg, M.D.**[4], **G. Petur Nielsen, M.D.**[4], **David G. Kirsch, M.D., Ph.D.**[8], **Edwin Choy, M.D., Ph.D.**[5], **David C Harmon, M.D.**[5], **Francis J. Hornicek, M.D., Ph.D.**[6], **Jonathan Dreyfuss, B.S.**[7], **Marek Ancukiewicz, Ph.D.**[2], **Dushyant V. Sahani, M.D.**[3], **Peter J. Park, Ph.D.**[7], **Rakesh K. Jain, Ph.D.**[2], and **Thomas F. DeLaney, M.D.**[2]

[1]Department of Surgery, Massachusetts General Hospital and Harvard Medical School Boston, MA

[2]Department of Radiation Oncology, Massachusetts General Hospital and Harvard Medical School Boston, MA

[3]Department of Radiology, Massachusetts General Hospital and Harvard Medical School Boston, MA

[4]Department of Pathology, Massachusetts General Hospital and Harvard Medical School Boston, MA

[5]Department of Medicine, Massachusetts General Hospital and Harvard Medical School Boston, MA

[6]Department of Orthopedic Surgery, Massachusetts General Hospital and Harvard Medical School Boston, MA

[7]Center for Biomedical Informatics at Harvard Medical School and the Partners Center for Personalized Genetic Medicine, Boston, MA

[8]Duke University Medical Center, Durham, NC, Departments of Radiation Oncology and Cancer Biology, University of Pennsylvania School of Medicine, Philadelphia, PA



[*]Corresponding author: Sam S. Yoon, M.D., Departments of Surgery, Massachusetts General Hospital, Yawkey 7B, 55 Fruit St., Boston, MA 02114, Tel: 617-726-4241, Fax: 617-724-3895, syoon@partners.org.

[9]These authors contributed equally to this article.



Presented at the American Society of Clinical Oncology Annual Meeting, Chicago, IL, on June 8, 2010.

**CONFLICT OF INTEREST NOTIFICATION**

Rakesh K. Jain
Advisory Role: SynDevRx, Millennium Pharmaceuticals, MorphoSys AG, Regeneron Pharmaceuticals, Genzyme, Dyax, AstraZeneca
Stock Ownership: SynDevRx
Honoraria: Genzyme, Alnylam
Research Funding: AstraZeneca, Dyax
David G. Kirsch
Advisory Role: Lumicell Diagnostics
Stock Ownership: Lumicell Diagnostics
All other authors reported no conflict of interest.

## Abstract

**Purpose—**Numerous preclinical studies demonstrate that angiogenesis inhibitors can increase the efficacy of radiation therapy (RT). We sought to examine safety and efficacy of bevacizumab (BV) and RT in soft tissue sarcomas (STS) and explore biomarkers for treatment response.

**Methods and Materials—**Patients (pts) with ≥5 cm, intermediate- or high-grade STS at significant risk of local recurrence (LR) received neoadjuvant BV alone followed by BV plus RT prior to surgical resection. Correlative science studies included analysis of serial blood and tumor samples as well as serial perfusion CT scans.

**Results—**The 20 pts had a median tumor size of 8.25 cm, with 13 extremity, 1 trunk, and 6 retroperitoneal/pelvis tumors. Neoadjuvant treatment was well tolerated with only 4 pts having grade 3 toxicities (hypertension, LFT elevation). BV plus RT resulted in ≥80% pathologic necrosis in 9 of 20 tumors (45%), which is over double the historical rate seen with RT alone. 3 pts had a complete pathologic response. Median microvessel density (MVD) decreased 53% after BV alone ($p<0.05$), and following combination therapy, median tumor cell proliferation decreased by 73%, apoptosis increased 10.4 fold, and blood flow, blood volume, and permeability surface area decreased by 62–72% ($p<0.05$). Analysis of gene expression microarrays of untreated tumors identified a 24-gene signature for treatment response. MVD and circulating progenitor cells at baseline and reduction in MVD and plasma soluble c-KIT with BV also correlated with good pathologic response ($p<0.05$). After a median follow-up of 20 months, only 1 patient had a LR.

**Conclusions—**This exploratory study indicates that BV increases the efficacy of RT against STS and may reduce LR, and thus this regimen warrants further investigation. Gene expression profiles and other tissue and circulating biomarkers show promising correlations with treatment response.



### Keywords

sarcoma; angiogenesis inhibitors; radiation therapy; bevacizumab; biomarkers


## INTRODUCTION

Soft tissue sarcomas (STS) are a heterogeneous group of tumors derived from cells usually of mesodermal origin. Local recurrence for primary STS after surgery alone can be as high as 33% for extremity tumors and 82% for retroperitoneal tumors (1;2). The addition of radiation therapy (RT) chas been prospectively demonstrated to decrease local recurrence for extremity and trunk tumors (1;3), and retrospective studies suggest RT may reduce local recurrence for retroperitoneal tumors (4;5). Despite aggressive surgery and RT, extremity and trunk tumors adjacent to vital structures and all retroperitoneal tumors still have a significant risk of local recurrence. The benefit of adjuvant chemotherapy in reducing local and distant recurrence rates is modest at best (6).

Vascular endothelial growth factor (VEGF) is over-expressed by most human cancers including STS and promotes tumor angiogenesis (7). Numerous preclinical studies have demonstrated that despite having anti-vascular effects, anti-VEGF therapies can improve the efficacy of RT (8;9), at least in part by vascular normalization (10). However human data on the mechanisms of action are sparse. Bevacizumab (BV) is a humanized anti-VEGF monoclonal antibody that binds VEGF and potently inhibits its activity (11). In a phase I/II study of 32 patients with rectal cancer, Willet *et al.* found that BV enhanced the effects of neoadjuvant chemoradiation resulting in a high pathologic response rate and no local recurrences (12;13). There are few clinical studies examining the combination of BV and RT without chemotherapy for solids tumors and none for STS.

Because the mechanisms of action of BV in solid tumors are far from clear and no biomarkers exist to stratify patients for BV therapy, research in this area remains a priority. To this end, we designed a study of neoadjuvant BV alone for two weeks, followed by BV with RT in patients with ≥5 cm, intermediate- or high-grade STS. Surgical resection of the tumor was performed 6–7 weeks after completion of neoadjuvant treatment. Perfusion CT scans were used to determine treatment effects on tumor blood flow and vascular permeability. Serial blood samples were analyzed to determine the effects of treatment on circulating angiogenic and cellular biomarkers. Tumor biopsies were obtained prior to treatment, after BV alone, and after combination therapy, and were analyzed for changes in gene and protein expression.

## METHODS AND MATERIALS

### Patients

This phase II trial was approved by the Dana-Farber/Harvard Cancer Center Institutional Review Board. Eligibility criteria included: histological diagnosis of primary STS or an isolated local recurrence of STS after prior surgery, at least 5 cm in size, intermediate- or high-grade, no metastatic disease, age ≥ 18 years, Zubrod performance status 0–2, and normal bone marrow, renal, and hepatic function. The following patients were excluded: clinically significant cardiovascular disease (e.g., uncontrolled hypertension >140/90, myocardial infarction, unstable angina), recent thromboembolic event, and hypercoagulable disorder. Informed written consent was obtained from all patients. Patients were accrued between 2006 and 2009, and median follow-up was 20 months.

### Study Treatment

Patients received 4 doses of BV (5 mg/kg) every 2 weeks (Fig. 1). RT was started with the second dose of BV to a total dose of 50.4 Gy in 28 fractions over 5.5 weeks. Specialized RT techniques included 3D conformal RT in 3 patients, intensity-modulated RT (IMRT) in 9 patients, proton beam RT (PBRT) in 1 patient, and combined IMRT and PBRT in 1 patient. Three patients with retroperitoneal tumors received intra-operative electron RT (IOERT) of 7.5–10 Gy to the posterior margin, and 3 patients with extremity/trunk tumors received a 10–16 Gy post-operative boost for a positive microscopic margin. Surgical resection of the tumor was performed 6–7 weeks following the completion of RT and 8–9 weeks after the last dose of BV. Blood samples were obtained before treatment and at weeks 2, 6, and 10. Tumor samples were obtained before treatment, at week 2, and at the time of surgery. Perfusion CT scans were performed before treatment, at week 2, and at week 10.

### Perfusion CT scans

Serial perfusion CT scans were performed to assess tumor vasculature (14;15). Scans were performed on a 16–64 slice multidetector row CT scanner (GE Healthcare). A 2–4 cm region of tumor was selected on noncontrast CT. Subsequently, dynamic CT of this region was performed for 45 seconds at the same table position immediately after initiation of intravenous infusion of 50–70cc of iodinated nonionic contrast media (Isovue 370, Bracco Diagnostics) at a rate of 7 ml/s. Delayed images of the tumor were obtained every 13 seconds for 4 minutes after the initiation of contrast. Data were analyzed on a workstation (Advantage Windows, GE) using commercially available CT perfusion 3.0 software, which implements a deconvolution approach to calculate regional blood flow (BF), blood volume (BV), mean transit time (MTT), and permeability-surface area (PS) product.

### Blood and biopsy tissue analysis

Blood samples were collected in EDTA-containing vacutainer tubes and spun at 1000 × g for 15 min, and plasma was separated and frozen in aliquots. Plasma samples were analyzed using multiplex array plates from Meso-Scale Discovery for serial measurements of VEGF, soluble VEGF receptors 1 (sVEGFR-1), placental growth factor (PlGF), basic fibroblast growth factor (bFGF), interleukin (IL)-6, IL-8, and tumor necrosis factor α (TNF-α). ELISA kits from R&D Systems were used for stromal cell-derived factor 1α (SDF-1α), soluble c-KIT, sVEGFR-2, and sVEGFR-3. All samples were run in duplicate. After amending the protocol, circulating progenitor cells (CPCs) and VEGFR-2 monocytes were phenotyped and enumerated by flow cytometric analyses of CD31, CD34, CD45, VEGFR2, and CD133 expression using a custom-made fluorescence-labeled monoclonal antibody cocktail (BD Pharmingen) and a standard protocol in fresh samples, using an LSR-II flow cytometer as previously described (16).

Two core needle (14–18G) tumor biopsies were performed in the same tumor regions, one prior to therapy and one 12 days after first BV infusion under image guidance (for deep tumors). CD31 and PCNA immunohistochemistry and TUNEL immunofluorescence were performed as previously described (17;18). Nuclei will be stained with Hoechst nuclear dye. Images were obtained on a Zeiss microscope and analyzed using AxioVision 4.0 software (Carl Zeiss Vision).

### Microarray analysis

RNA was isolated from tumor tissue using the Qiagen RNeasy kit (Qiagen, Valencia, CA). RNA quality was assessed using 2100 Bioanalyzer (Agilent, Palo Alto, CA) and amplification was performed using the Illumina TotalPrep RNA Amplification Kit (Illumina, San Diego, CA). Amplified cRNAs was hybridized on HumanRef-8 Expression BeadChips, which targets over 24,000 known genes (Illumina). Image analysis was carried out using Illumina's BeadStudio v3.0.14 Gene Expression Module. All statistical analyses were conducted using the statistical software R (http://www.r-project.org). For unsupervised clustering, we selected the most variable 1000 genes based on coefficient of variation (CV). Agglomerative hierarchical clustering was performed using $1 - r$ (Pearson correlation) as the distance measure with average linkage. We also used another variability measure of median absolute deviation (MAD) and tested different number of highly variable genes ($n = 200$ to 2000), observing similar discriminating pattern between responders and non-responders (data not shown). To identify genes whose expression is associated with drug responsiveness, we used the *t*-test to rank the genes and used the top $M$ genes in the K-nearest neighbors (KNN) class prediction method (PMID: 10521349). The prediction model performed well in a leave-one-out cross validation across a range of predictor size $M$, with accuracy of 93.8% (15 true predictions out of 16 leave-one-out tests). We report 24 genes that appeared more than at least 15 times out of the 16 tests in a predictor size of $M = 50$ genes. We note that one sample (#7) was misclassified in the leave-one-out cross validation, but it was correctly classified as a 'responder' in hierarchical clustering using the 24-gene signature. Four samples were excluded because on histological examination, a significant amount of non-tumor tissue was present or RNA isolation was inadequate.

### Data and Statistical Analyses

The primary objective of this study was to determine the pathologic response rate of neoadjuvant BV combined with RT. Pathologic response of ≥80% necrosis after pre-operative RT alone for STS is about 15% (19). We considered neoadjuvant BV to be a worthwhile addition to this pre-operative regimen if the true response rate improved to 30%.

Comparison of potential biomarkers versus pretreatment values were performed for all variables using Wilcoxon exact test for paired data, after adjusting the p values for multiple comparisons over different time points. Comparisons of variables for different subgroups were performed using the Wilcoxon two-sample test. Correlations were quantified using Kendall's tau coefficients. In these exploratory, hypothesis-generating studies, the concern was to avoid both false positive and false negative results for association with outcome. Since the parameters measured were not random but rather mechanism-based biomarkers, we did not adjust for multiple statistical tests for different biomarkers.

## RESULTS

### Patient and tumor characteristics

The treatment regimen along with timing of blood and tumor samples and perfusion CT scans are shown in Fig. 1. The patient and tumor characteristics for all 20 patients as well as for the 14 patients with extremity/truncal tumors versus the 6 patients with retroperitoneal/pelvic tumors are outlined in Table 1. Median tumor size was 8.25 cm. The most common histologic subtypes were fibroblastic sarcoma, liposarcoma, and leiomyosarcoma. Eighteen of 20 tumors (90%) were intermediate- or high-grade. The two patients with low-grade tumors were entered onto the study because their initial biopsy was read as intermediate or high-grade, but the final pathology on the surgical specimen was changed to low-grade.

### BV and RT

Ninety percent of patients received all 4 doses of BV. One patient had two doses held for grade 3 hypertension and LFT elevations, and another patient had 1 dose held for grade 3 LFT elevations. The dose, type, and timing of RT are summarized in Table 2. All patients received 50.4 Gy of preoperative RT, and 6 patients received either intra-operative RT or postoperative RT (7.5–16 Gy) for a close or positive margin. RT was delivered using a variety of techniques including IMRT and proton beam RT. Selected adverse events are summarized in Table 3. During treatment with BV and RT, there were no grade 4 toxicities, and 4 pts (20%) had grade 3 toxicities (hypertension, LFT elevation). Common toxicities included fatigue (n=11), hypertension (10), anorexia and nausea (9), and radiation dermatitis (8). One patient developed a 5 mm ulceration overlying a superficial ankle tumor following core biopsy, and this ulceration healed prior to surgery. There were no episodes of tumor bleeding, bowel perforation or fistula, or thromboembolic event.

### Surgical treatment and complications

Tumors were resected 8–9 weeks after the last dose of BV and 6–7 weeks after the last dose of RT. Of the 13 patients with extremity tumors, 12 underwent resection with limb preservation and one patient required amputation. Three patients required muscle or fasciocutaneous flaps for closure. Of the 6 patients with retroperitoneal or pelvic tumors, all required resection of the tumor along with contiguous organs including colon (n=3), kidney (3), spleen, pancreas, testicle/cord, major vessel or nerve, and bone (all n=1). Following complete pathological analysis, 15 patients (75%) had negative microscopic margins, 4 had microscopically positive margins (20%), and one (5%) had a small amount of gross residual disease left *in situ*. Median length of stay was 5 days (range 1–15 days). Major post-operative complications occurred in 5 patients (25%) and included 4 major wound complications and one pulmonary embolism. The wound complication rate is comparable with that seen with neoadjuvant RT alone (20).

### Efficacy by histological and radiological analysis

Following neoadjuvant BV and RT, 9 tumors (45%) had ≥80% pathologic necrosis. Of these 9 tumors, 2 had 80–89% necrosis, 4 had 90–99% necrosis, and 3 had 100% necrosis (i.e. pathologic complete response). Of note, a recent analysis of the MGH RT Sarcoma Database and review of the literature demonstrated that <20% of STS receiving neoadjuvant RT alone have ≥80% pathologic necrosis and complete necrosis is rare (19).

Perfusion CT scans were used to assess blood flow, blood volume, mean transit time, and permeability surface area based on previously published algorithms (14;15). Certain tumors demonstrated significant reductions in perfusion CT parameters with BV alone. Figure 2A demonstrates the perfusion CT scans from patient with ankle synovial STS before and 10 days after a single dose of BV. Blood volume and permeability surface area were both reduced by 38%. For all patients, there was wide heterogeneity in the pretreatment values of perfusion CT parameters, and no statistically significant decrease in any parameter following BV alone (Fig. 2B). However, median blood flow, blood volume, and permeability surface area significantly decreased by 62–72% following combination treatment with BV and RT.

Lack of correlation between radiological response and pathological response has been previously demonstrated for STS (21). Based on RECIST criteria, 3 patients had a partial response (≥30% decrease in diameter), 14 patients had stable disease, and 3 patients had progressive disease (≥20 increase in diameter). For the 3 patients with a partial response, all tumors had 95–100% necrosis. However, 2 of the 3 patients with progressive disease by RECIST criteria had tumors with 95% necrosis and the other patient had 65% necrosis.

Tumors were assessed for proliferation, apoptosis, and microvessel density using immunohistochemical methods for PCNA, TUNEL, and CD31, respectively. There was a wide range in baseline proliferation and apoptosis rates between tumors, and following BV alone, there were no significant changes in median tumor cell proliferation or apoptosis (Fig. 3A, B, D). After combination therapy, median proliferation decreased by 73% and median apoptosis increased 10.2 fold. In contrast to tumor cell proliferation and apoptosis, median microvessel density was significantly decreased by 53% with BV alone (Fig. 3C, D). Furthermore, a high pretreatment microvessel density significantly correlated with tumor necrosis following combination therapy (tau=0.53, p=0.0031), and the decrease in microvessel density after BV alone also correlated with tumor necrosis (tau=0.43, p=0.0154).

### Local and distant recurrence

After a median follow-up of 24 months, one patient suffered a local recurrence (Fig. 4A). This one patient was a 75 year-old man who presented with a 20.2 cm LR of a well-differentiated and de-differentiated liposarcoma of the RP. Following pre-operative treatment with BV and RT (IMRT and PBRT), the patient underwent surgical resection with some residual disease left in situ on the duodenum and IVC. This gross residual disease received 10 Gy of IOERT. This residual gross disease began to grow 16 months following surgery. All other patients had no evidence of LR. At the time of last follow-up, seven patients had suffered a distant recurrence to the lungs (n=7), brain (2), regional nodes (2), soft tissue (1), stomach (1), and/or liver (1). Median time to distant recurrence was 7 months (range 2–36 months) (Fig. 4B). When stratified by tumor location, only patients with extremity/truncal tumors had distant recurrences; none of the patients with RP/pelvis tumors had a distant recurrence (Fig. 3C).

### Gene expression microarrays

Tumor samples prior to treatment were analyzed using oligonucleotide microarrays. Unsupervised hierarchical clustering for global gene expression patterns was performed based on the 1000 most variable genes (Fig. 5A). Some histologic subtypes including two pleomorphic fibroblastic sarcomas and two well-differentiated/dedifferentiated liposarcomas clustered together, while other histologic subtypes including three fibroblastic/myofibroblastic sarcomas and three leiomyosarcomas did not all cluster together. Interestingly in this unsupervised analysis, all tumors with ≥80% pathologic necrosis clustered in one group while all tumors with <80% pathologic necrosis clustered in second group with 100% accuracy. Further analysis resulted in a 24-gene signature which differentiated tumors with good response versus poor response (Fig. 5B). Detailed information on differentially expressed genes is listed in Suppl. Table 1.

### Circulating biomarkers

Median plasma VEGF concentration rose 6–7-fold after BV alone and after BV with RT ($p<0.0001$) (Table 4). Similarly, PlGF concentration increased 2-fold throughout the neoadjuvant treatment ($p<0.0001$). Surprisingly, we also found significantly decreased sVEGFR-3 plasma concentration over the course of therapy ($p<0.01$). Levels of bFGF, sVEGFR-1, sVEGFR-2, IL-6, IL-8, TNF-α, SDF-1α, soluble c-KIT, CPCs, and VEGFR-2-positive monocytes did change significantly during treatment (Suppl. Table 2). Increased tumor necrosis correlated with higher numbers of $CD34^{+}CD45^{+}$ CPCs prior to therapy (tau test= −0.57, p=0.017), and with a decrease in plasma concentration of soluble c-KIT at day 14 after BV alone (tau=−0.52, p=0.017).

## DISCUSSION

In this study of patients with ≥5 cm, intermediate- or high-grade STS, the addition of neoadjuvant BV to RT led to no grade IV toxicities and grade III toxicities in only 4 patients. Neoadjuvant BV and RT resulted in ≥80% necrosis in 45% of tumors, which is over double the historical rate with RT alone. Thus this treatment regimen appears be safe and to increase the degree of pathologic necrosis compared to RT alone.

Despite numerous preclinical studies demonstrating that VEGF inhibitors augment the efficacy of RT, this is the first clinical trial combining BV with RT for STS. Very few clinical trials have evaluated a specific anti-angiogenic agent combined with RT and no chemotherapy. One study treated patients with a variety of solid tumors with angiostatin at increasing doses and RT for a minimum of 25 fractions (22). There were 17 evaluable patients, no added toxicity was observed in normal tissue within the RT field, and tumor response was noted in 90% of patients. In another study, 25 patients with recurrent gliomas were treated with BV and RT with an overall response rate of 50% and median overall survival of 12.5 months (23). In addition to the rectal cancer studies described earlier (12;13), Koukourakis *et al.* found that BV combined with amifostine, capecitabine, and conformal hypofractionated RT caused a complete response in 13 of 19 (69%) of evaluable patients with locally advance inoperable colorectal cancer (13;24). Crane *et al.* examined BV combined with capecitabine and RT in a phase I trial for locally advanced pancreatic cancer. (25). Twenty percent of patients had a partial response, and median survival was 11.6 months. These studies illustrate the broad variations in response that solid tumors may have to the combination of BV and RT, with or without chemotherapy.

All untreated tumors were analyzed using gene expression microarrays. Unsupervised hierarchical clustering of STS has found clustering of histologic subtypes in some studies (26) but not in others (27). Gene signatures have been developed that are associated with

extent of disease, metastasis, and response to treatment (26). We previously demonstrated that histological subtypes of STS often share expression of angiogenesis-related genes, and these expression patterns are distinct from normal tissues (7). In this study, gene expression analysis of STS samples prior to treatment revealed that good responders and poor responders had global gene expression patterns that were significantly different. Thus global gene expression patterns or a more limited gene expression profile may be accurate means of discriminating tumors which will respond well to this therapy.

Our correlative science studies were designed to examine the effects of BV alone and BV with RT on tumors and tumor vasculature. Given the wide heterogeneity that is inherent in STS, we found significant variations in STS in terms of tumor proliferation, apoptosis, microvessel density, circulating proteins and cells, and perfusion CT parameters. BV alone significantly decreased tumor microvessel density by about 50% without decreasing blood flow rate, suggesting that the function of remaining tumor vasculature was not impaired but rather improved by BV, consistent with vascular normalization (10;13). Moreover, combination therapy significantly decreased tumor proliferation and increased apoptosis. As seen in studies of anti-angiogenic agents in other tumors, we show that VEGF blockade with BV increased plasma VEGF and PlGF concentration in STS patients (28). Moreover, we found that BV treatment decreased plasma concentration of sVEGFR3 (a receptor for VEGF-C and -D but not for VEGF or PlGF) suggesting a previously unrecognized indirect effect of VEGF blockade on this pathway in cancer patients. No validated biological markers currently exist for appropriately selecting cancer patients for anti-angiogenic therapy, although several biomarkers have been identified from examination of host factors, blood, tumor tissue, and radiological studies (28). The promising but preliminary associations found in this trial should be further explored in larger studies.

In summary, the results of this single arm, phase II study of neoadjuvant BV and RT for STS show encouraging activity and safety, and support the further evaluation of this regimen. Local recurrence occurred in only one patient. Using a large battery of correlative science studies, we found that BV alone can decrease microvessel density in tumors, and that combining BV with RT increased the degree of tumor necrosis and tumor cell apoptosis, and reduced tumor cell proliferation, blood flow, blood volume, and permeability. Several potential biomarkers for response to therapy, including gene expression profiles, were identified. These mechanistic insights and the potential response biomarkers identified in this study require further validation.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## Acknowledgments

This study was supported by NIH/NCI grants 5 K12 CA 87723-03 (S.S.Y.), 1 R21 CA117128-01 (S.S.Y.), and U54 LM008748 (P.J.P.), Society for Surgical Oncology Clinical Investigator Award (S.S.Y.), R01 CA115767 and P01 80124 (R.K.J.), and Ira J. Spiro Translational Research Award (D.G.D.).

## REFERENCES

1. Yang JC, Chang AE, Baker AR, et al. Randomized prospective study of the benefit of adjuvant radiation therapy in the treatment of soft tissue sarcomas of the extremity. J Clin Oncol. 1998 Jan; 16(1):197–203. [PubMed: 9440743]
2. Pisters PW, O'Sullivan B. Retroperitoneal sarcomas: combined modality treatment approaches. Curr Opin Oncol. 2002 Jul; 14(4):400–405. [PubMed: 12130924]

3. Pisters PW, Harrison LB, Woodruff JM, et al. A prospective randomized trial of adjuvant brachytherapy in the management of low-grade soft tissue sarcomas of the extremity and superficial trunk. J Clin Oncol. 1994 Jun; 12(6):1150–1155. [PubMed: 8201376]
4. Pawlik TM, Pisters PW, Mikula L, et al. Long-Term Results of Two Prospective Trials of Preoperative External Beam Radiotherapy for Localized Intermediate- or High-Grade Retroperitoneal Soft Tissue Sarcoma. Ann Surg Oncol. 2006 Feb 24; 13(4):508–517. [PubMed: 16491338]
5. Yoon SS, Chen YL, Kirsch DG, et al. Proton-Beam, Intensity-Modulated, and/or Intraoperative Electron Radiation Therapy Combined with Aggressive Anterior Surgical Resection for Retroperitoneal Sarcomas. Ann Surg Oncol. 2010 Feb 12. epub Feb 12, 2010.
6. Schuetze SM, Patel S. Should patients with high-risk soft tissue sarcoma receive adjuvant chemotherapy? Oncologist. 2009 Oct; 14(10):1003–1012. [PubMed: 19808770]
7. Yoon SS, Segal NH, Park PJ, et al. Angiogenic profile of soft tissue sarcomas based on analysis of circulating factors and microarray gene expression. J Surg Res. 2006 Oct; 135(2):282–290. [PubMed: 16603191]
8. Kozin SV, Boucher Y, Hicklin DJ, et al. Vascular endothelial growth factor receptor-2-blocking antibody potentiates radiation-induced long-term control of human tumor xenografts. Cancer Res. 2001 Jan 1; 61(1):39–44. [PubMed: 11196192]
9. Wachsberger P, Burd R, Dicker AP. Tumor response to ionizing radiation combined with antiangiogenesis or vascular targeting agents: exploring mechanisms of interaction. Clin Cancer Res. 2003 Jun; 9(6):1957–1971. [PubMed: 12796357]
10. Winkler F, Kozin SV, Tong RT, et al. Kinetics of vascular normalization by VEGFR2 blockade governs brain tumor response to radiation: role of oxygenation, angiopoietin-1, and matrix metalloproteinases. Cancer Cell. 2004 Dec; 6(6):553–563. [PubMed: 15607960]
11. Chen HX. Expanding the clinical development of bevacizumab. Oncologist. 2004; 9 Suppl 1:27–35. [PubMed: 15178813]
12. Willett CG, Boucher Y, di Tomaso E, et al. Direct evidence that the VEGF-specific antibody bevacizumab has antivascular effects in human rectal cancer. Nat Med. 2004 Feb; 10(2):145–147. [PubMed: 14745444]
13. Willett CG, Duda DG, di TE, Boucher Y, et al. Efficacy, safety, and biomarkers of neoadjuvant bevacizumab, radiation therapy, and fluorouracil in rectal cancer: a multidisciplinary phase II study. J Clin Oncol. 2009 Jun 20; 27(18):3020–3026. [PubMed: 19470921]
14. Kambadakone AR, Sahani DV. Body perfusion CT: technique, clinical applications, and advances. Radiol Clin North Am. 2009 Jan; 47(1):161–178. [PubMed: 19195541]
15. Sahani DV, Kalva SP, Hamberg LM, et al. Assessing tumor perfusion and treatment response in rectal cancer with multisection CT: initial observations. Radiology. 2005 Mar; 234(3):785–792. [PubMed: 15734934]
16. Duda DG, Cohen KS, Scadden DT, et al. A protocol for phenotypic detection and enumeration of circulating endothelial cells and circulating progenitor cells in human blood. Nat Protoc. 2007; 2(4):805–810. [PubMed: 17446880]
17. Detwiller KY, Fernando NT, Segal NH, et al. Analysis of hypoxia-related gene expression in sarcomas and effect of hypoxia on RNA interference of vascular endothelial cell growth factor A. Cancer Res. 2005 Jul 1; 65(13):5881–5889. [PubMed: 15994966]
18. Ryeom S, Baek KH, Rioth MJ, et al. Targeted deletion of the calcineurin inhibitor DSCR1 suppresses tumor growth. Cancer Cell. 2008 May; 13(5):420–431. [PubMed: 18455125]
19. Canter RJ, Khatri VP, Wilton M, et al. Radiographic and histologic response to neoadjuvant radiotherapy in patients with soft tissue sarcoma. Ann Surg Oncol. 2009 Feb 1.16 Supplement 1:117.
20. O'Sullivan B, Davis AM, Turcotte R, et al. Preoperative versus postoperative radiotherapy in soft-tissue sarcoma of the limbs: a randomised trial. Lancet. 2002 Jun 29; 359(9325):2235–2241. [PubMed: 12103287]
21. Stacchiotti S, Collini P, Messina A, et al. High-grade soft-tissue sarcomas: tumor response assessment--pilot study to assess the correlation between radiologic and pathologic response by using RECIST and Choi criteria. Radiology. 2009 May; 251(2):447–456. [PubMed: 19261927]

22. Dicker AP, Anne R, Bonanni R, et al. Phase I trial results of recombinant human angiostatin protein (rhA) and external beam radiation therapy (EBRT). 38th Annual Meeting of the American Society for Clinical Oncology. 2002 [abstract].

23. Gutin PH, Iwamoto FM, Beal K, et al. Safety and efficacy of bevacizumab with hypofractionated stereotactic irradiation for recurrent malignant gliomas. Int J Radiat Oncol Biol Phys. 2009 Sep 1; 75(1):156–163. [PubMed: 19167838]

24. Koukourakis MI, Giatromanolaki A, Sheldon H, et al. Phase I/II trial of bevacizumab and radiotherapy for locally advanced inoperable colorectal cancer: vasculature-independent radiosensitizing effect of bevacizumab. Clin Cancer Res. 2009 Nov 15; 15(22):7069–7076. [PubMed: 19887481]

25. Crane CH, Ellis LM, Abbruzzese JL, et al. Phase I trial evaluating the safety of bevacizumab with concurrent radiotherapy and capecitabine in locally advanced pancreatic cancer. J Clin Oncol. 2006 Mar 1; 24(7):1145–1151. [PubMed: 16505434]

26. Tschoep K, Kohlmann A, Schlemmer M, et al. Gene expression profiling in sarcomas. Crit Rev Oncol Hematol. 2007 Aug; 63(2):111–124. [PubMed: 17555981]

27. Nagayama S, Katagiri T, Tsunoda T, et al. Genome-wide analysis of gene expression in synovial sarcomas using a cDNA microarray. Cancer Res. 2002 Oct 15; 62(20):5859–5866. [PubMed: 12384549]

28. Jain RK, Duda DG, Willett CG, et al. Biomarkers of response and resistance to antiangiogenic therapy. Nat Rev Clin Oncol. 2009 Jun; 6(6):327–338. [PubMed: 19483739]

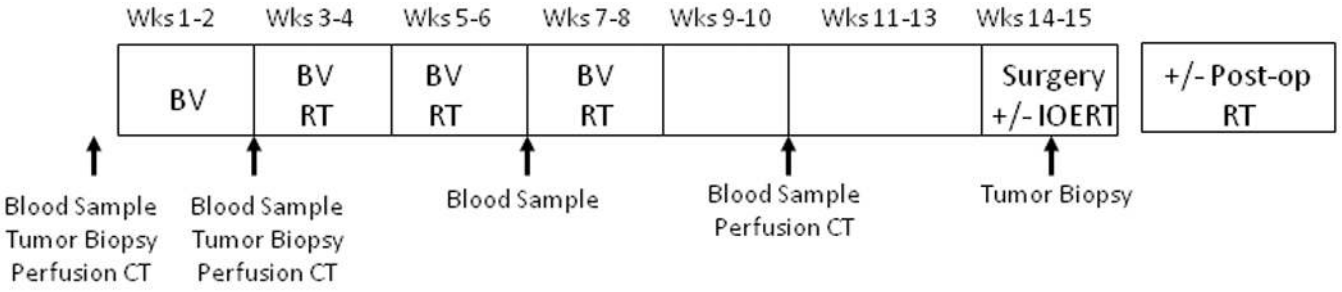


**Figure 1.**

Schema for clinical trial and perfusion CT scan parameters. Patients prior to surgical resection received an initial dose of BV followed two weeks later by a 6-week course of BV combined with RT. Surgical resection (Surgery) of the tumor was then performed 6–7 weeks after completion of neoadjuvant treatment. Intra-operative electron RT (IOERT) or post-operative RT (post-op RT) was delivered when indicated. Blood sample, tumor biopsy, and perfusion CT scans were obtained where indicated.

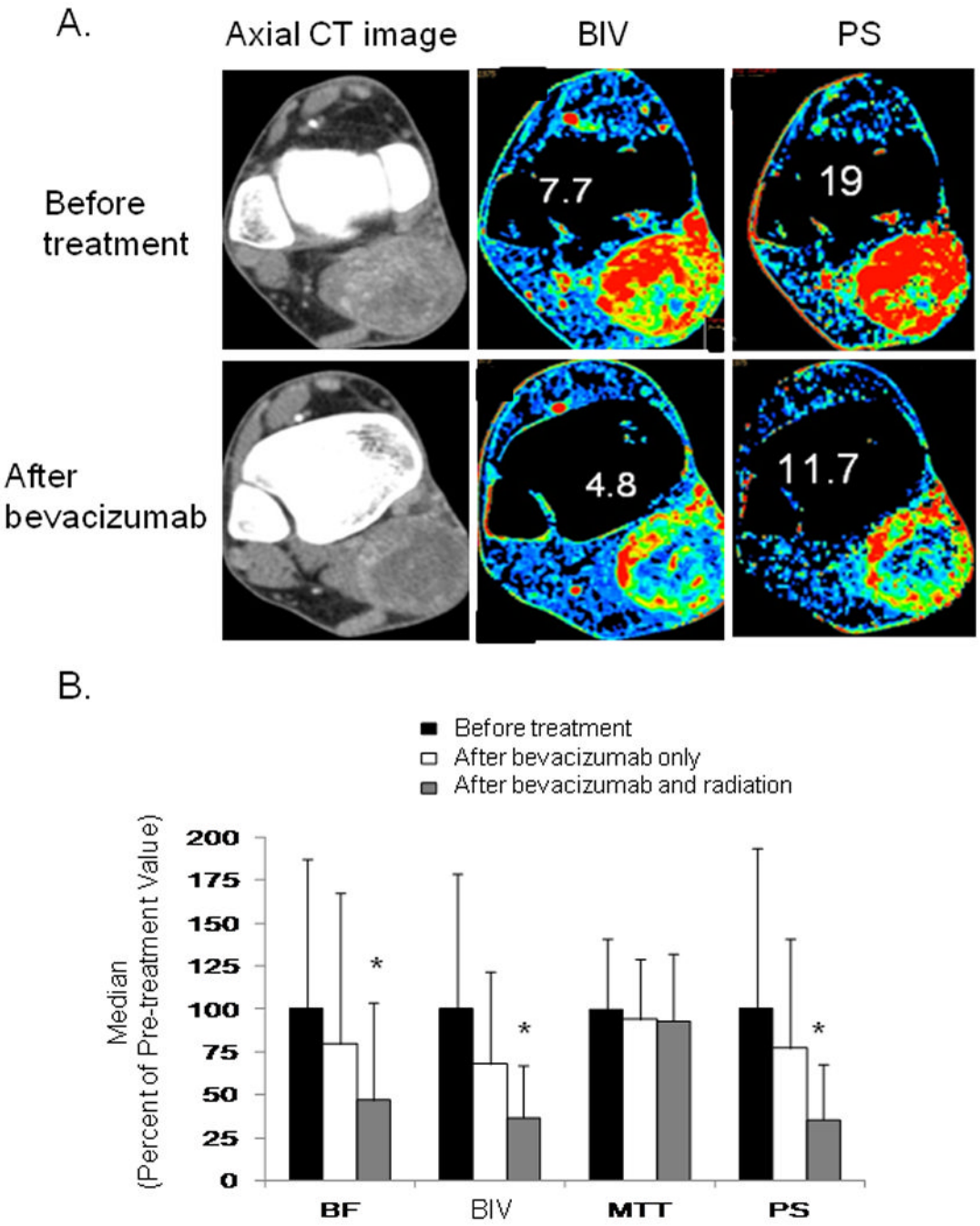


**Figure 2.**

Perfusion CT scans. (A) Perfusion CT scans from patient #2. Axial CT scan images and post-processing images of blood volume (BIV) and permeability surface area (PS) of an ankle synovial sarcoma before and after one dose of BV. (B) Perfusion CT scan median values of blood flow (BF), blood volume (BV), mean transit time (MTT), and permeability surface area (PS) are shown normalized to pre-treatment values. Bars represent standard deviation, *p<0.05.

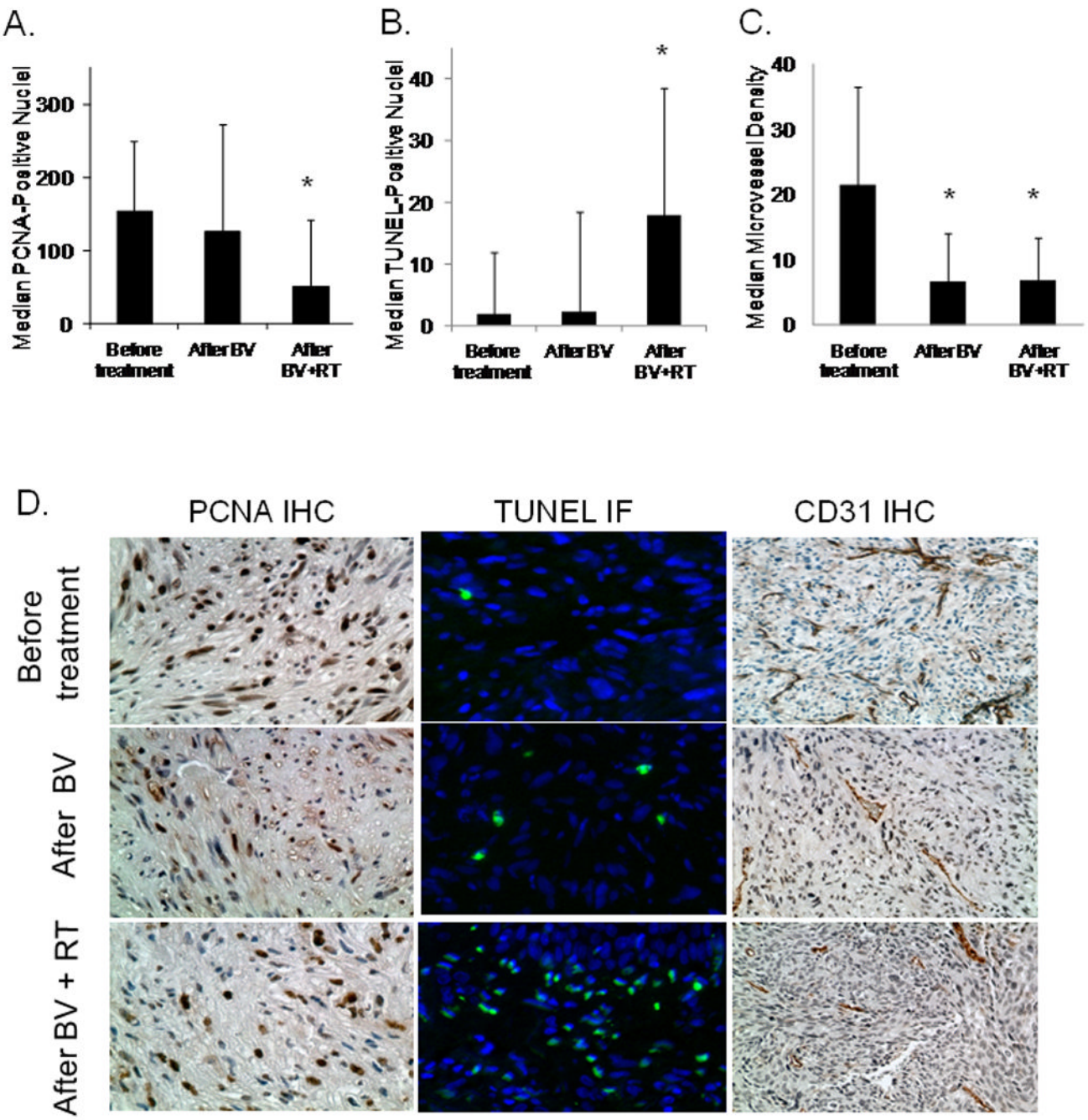


**Figure 3.**

Proliferation, apoptosis, and microvessel density. (A) Median number of proliferating cells as measured by immunohistochemistry for proliferation cell nuclear antigen (PCNA) before treatment, after BV and after BV plus RT. (B) Median number of apoptotic cells as measured by TUNEL staining. (C) Median microvessel density following staining for CD31. (D) Representative images of tumor sections for PCNA immunohistochemistry (IHC), TUNEL immunofluorescence (IF), or CD31 IHC. Bars represent standard deviation, *p<0.05.

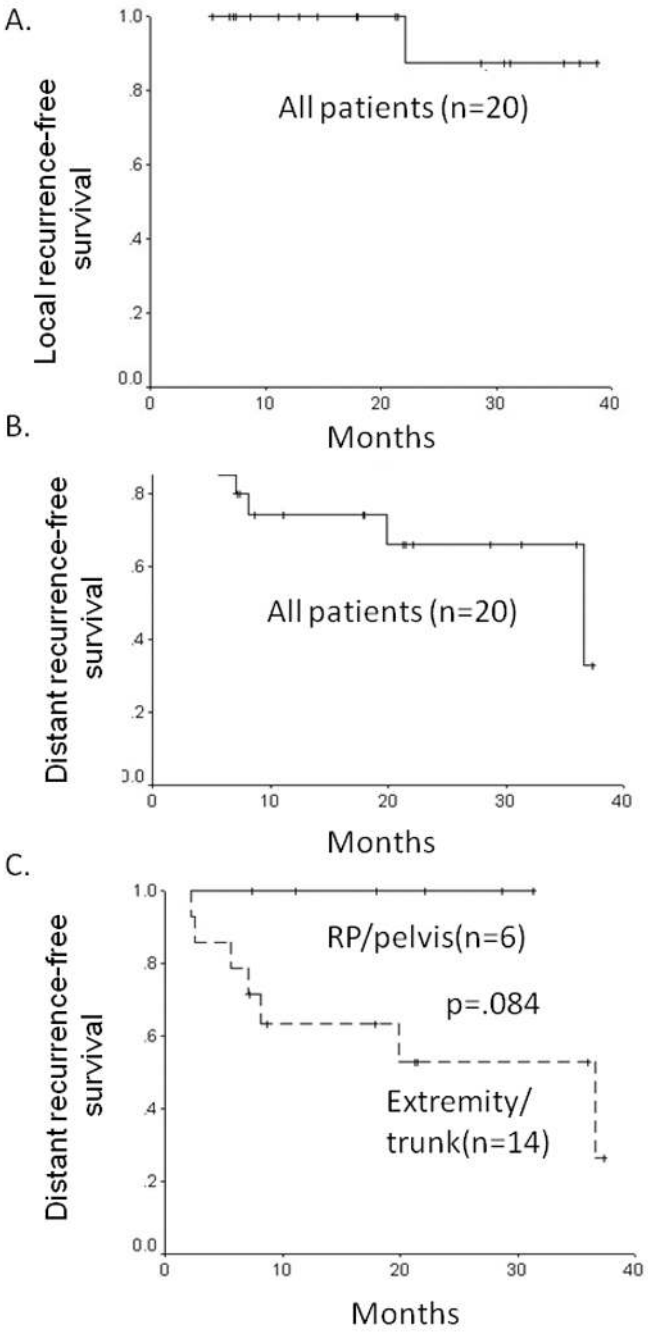


**Figure 4.**
Local and distant recurrence-free survival. (A) LR-free survival. Distant recurrence-free survival for all patients (B) or stratified by tumor location (C).

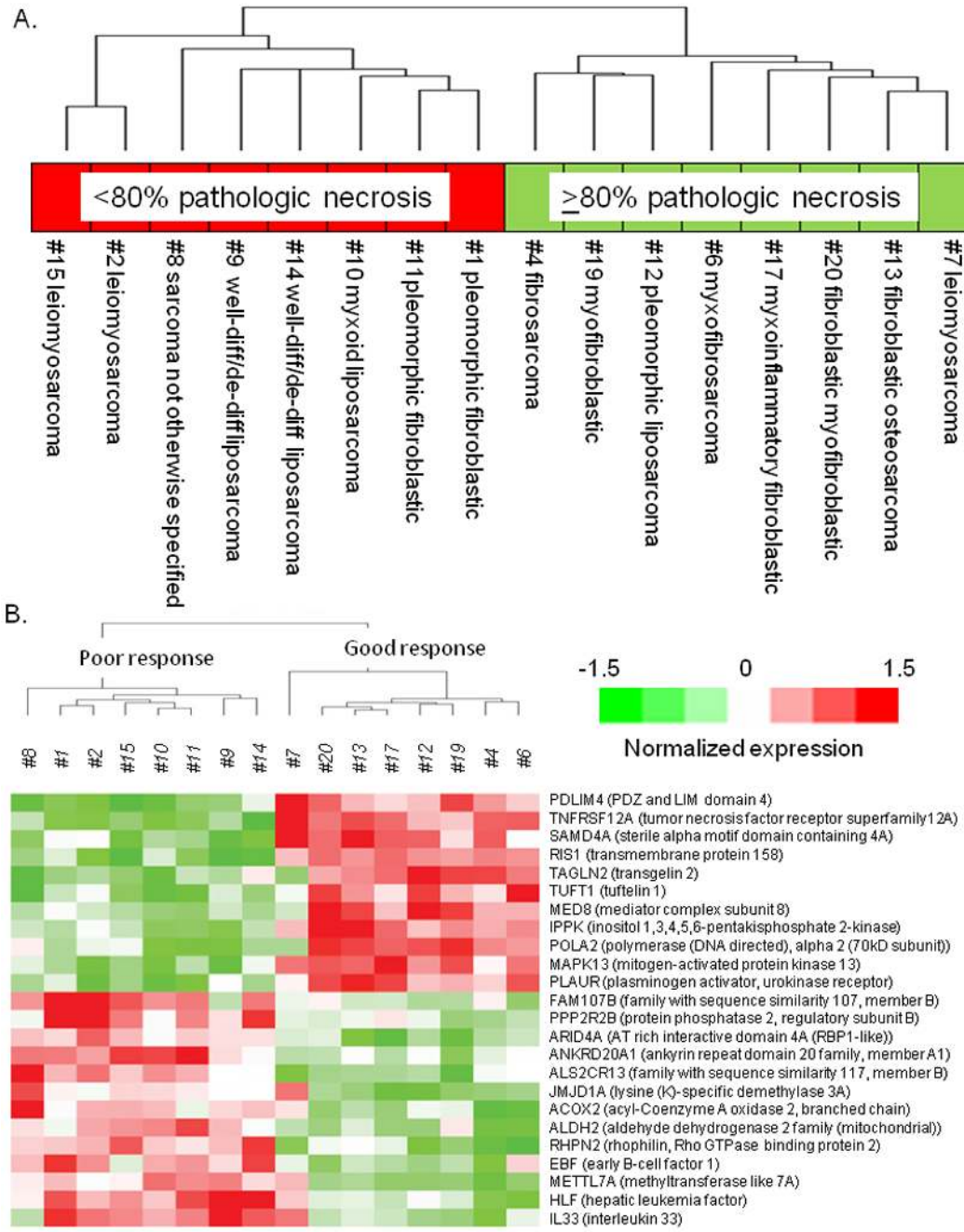


**Figure 5.**

Gene expression microarrays. (A) Unsupervised hierarchical clustering of pre-treatment tumor samples. Histologic subtype and pathologic necrosis following treatment is given for each sample. (B) Supervised hierarchical clustering based on 24 genes which were found to distinguish good responders (>80% necrosis) from poor responders (<80% necrosis). Patient number given for each sample. Color bar shows the extent of normalized expression intensities in heat map.

## TABLE 1

Patient and Tumor Characteristics

| | All patients | Extremity (n=13) and Trunk (n=1) | Retroperitoneum (n=4) and pelvis (n=2) |
|---|---|---|---|
| Number | 20 | 14 | 6 |
| Median age (range) | 59 (26–75) | 56 (26–70) | 62 (46–75) |
| Female gender | 7 (35%) | 4 (28.6%) | 3 (50%) |
| Symptoms | 8 (40%) | 5 (35.7%) | 3 (50%) |
| Palpable Mass | 17 (85%) | 14 (100%) | 3 (50%) |
| Median size in cm (range) | 8.25 (5–20.2) | 7.3 (5–15) | 15.2 (7.8–20.2) |
| Histology | | | |
| Fibroblastic | 8 (40%) | 8 (57%) | 0 |
| Liposarcoma | 6 (30%) | 2 (14.3%) | 4 (66.7%) |
| Leiomyosarcoma | 4 (20%) | 2 (14.3%) | 2 (33.3%) |
| Fibroblastic osteosarcoma | 1 (5%) | 1 (7.1%) | 0 |
| NOS | 1 (5%) | 1 (7.1%) | 0 |
| Grade | | | |
| Low | 2 (10%) | 0 | 2 (33.3%) |
| Intermediate | 8 (40%) | 7 (50%) | 1 (16.7%) |
| High | 10 (50%) | 7 (50%) | 3 (50%) |

## TABLE 2

Radiation Therapy

| | All patients | Extremity (n=13) and Trunk (n=1) | Retroperitoneum (n=4) and pelvis (n=2) |
|---|---|---|---|
| Number | 20 | 14 | 6 |
| External beam radiation median dose (range) | 50.4 Gy (50.4–66.4) | 50.4 Gy (50.0–66.4) | 54.2 (50.4–60.4) |
| Type of external beam radiation | | | |
| Standard | 5 (25%) | 4 (28.6%) | 1 (16.7%) |
| 3D conformal | 3 (15%) | 3 (21.4%) | 0 |
| Intensity-modulated | 9 (45%) | 7 (50%) | 2 (33.3%) |
| Proton beam | 1 (5%) | 0 | 1 (16.7%) |
| Both proton beam and intensity-modulated | 2 (10%) | 0 | 2 (33.3%) |
| Timing of radiation | | | |
| Preoperative | 20 (100%) | 14 (100%) | 6 (100%) |
| Intra-operative | 3 (15%) | 0 | 3 (50%) |
| Postoperative | 3 (15%) | 3 (21.4%) | 0 |

## TABLE 3

Selected Adverse Events

| | Grade | | |
|---|---|---|---|
| **Adverse Event** | **1** | **2** | **3** |
| Hypertension | 3 | 5 | 2 |
| Proteinurnia | 3 | 1 | |
| Radiation dermatitis | 5 | 3 | |
| Fatigue | 6 | 5 | |
| *Ulceration | 1 | | |
| Nose hemorrhage | 3 | | |
| Anorexia/nausea/vomiting | 7 | 2 | |
| Diarrhea | 6 | | |
| Constipation | 3 | | |
| LFT elevation | 3 | | 2 |
| Dehydration | 1 | | |

*Tumor ulceration at size of core biopsy

Note: No tumor bleeding, bowel perforation/fistula, or thromboembolic events

## Table 4

Circulating biomarkers with significant changes

| Biomarker | Pre-treatment | Day 14 BV | Day 24 BV+RT | Day 64 Post-treatment |
|---|---|---|---|---|
| Plasma PlGF | 19.5 [14.7–22.4]* | 29.7 [26.9–39.7] | 40.8 [31.7–45.6] | 33.8 [25.9–40.2] |
| (pg/ml) | (N=18) | (N=20) | (N=19) | (N=18) |
| p-value | N/A | <0.0001 | <0.0001 | 0.0002 |
| Plasma VEGF | 167 [86–282] | 1106 [880–1255] | 1550 [1039–1789] | 1287 [970–1500] |
| (pg/ml) | (N=18) | (N=20) | (N=19) | (N=18) |
| p-value | N/A | <0.0001 | <0.0001 | <0.0001 |
| Plasma sVEGFR3 | 494 [362–792] | 250 [149–380] | 205 [99–334] | 224 [129–394] |
| (pg/ml) | (N=19) | (N=20) | (N=19) | (N=18) |
| p-value | N/A | 0.0023 | 0.0013 | 0.0035 |

* Data are shown as medians and inter-quartile ranges.

P-values are multiple-comparison adjusted p-values from the paired exact Wilcoxon test.

BV, bevacizumab; RT, radiation therapy.