

RADIATION THERAPY ONCOLOGY GROUP

RTOG S-0120

A RANDOMIZED PHASE I/II STUDY OF PREOPERATIVE RADIOTHERAPY WITH/WITHOUT SUGEN 5416 (NSC #696819; A TK INHIBITOR ANTI-ANGIOGENESIS COMPOUND) IN THE MANAGEMENT OF LOW TO INTERMEDIATE GRADE SOFT TISSUE SARCOMA OF THE TRUNK OR EXTREMITY

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SCHEMA

R		R	<u>Phase I*</u>	R	<u>Phase II</u>
E	<u>Tumor Status</u>	E		A	
C	1. Primary	G	Dose Escalation (<i>See 7.1.2 and 13.3.2</i>)	N	
O	2. Recurrent	I		D	<u>Group 5:</u> RT and Sugén 145 mg/m ²
R		S	<u>Group 1:</u> RT and Sugén 110 mg/m ²	O	
D		T	<u>Group 2:</u> RT	M	<u>Group 6:</u> RT
		E	<u>Group 3:</u> RT and Sugén 145 mg/m ²	I	
		R	<u>Group 4:</u> RT	Z	
				E	

Radiation therapy for all groups: 50 Gy (2.0 Gy x 5 days x 5 weeks).

Group 1: RT and Sugén 5416 at 110 mg/m² twice weekly during RT. Give 4 to 8 doses after RT is completed, twice weekly for next 2 to 4 weeks.

Group 3 and Group 5: RT and Sugén 5416 at 145 mg/m² twice weekly during RT. Give 4 to 8 doses after RT is completed, twice weekly for next 2 to 4 weeks.

*Patients with positive microscopic or macroscopic margins and less than 100% tumor necrosis after protocol surgery will receive post-operative radiation treatment to 16 Gy in eight daily fractions; SU5416 should be administered at pre-operative tolerated dose with post-operative radiation for 3 doses on Days 2, 4, and 9.

All patients have surgery per Section 8.0 within 10 weeks of RT start.

Eligibility: (*See Section 3.0 for details*)

- Histologically confirmed, locally confined, soft tissue sarcoma, low or intermediate grade (*G1/2*) and measures > 5cm; located on upper or lower extremities or body wall.
- AJCC (1998) G1 or G2 (*on a 1-4 scale*), T₂a or T₂b (*Stage IB, IIA*).
- Treatment must begin within two weeks after registration.
- No evidence of metastases.
- No prior chemotherapy, irradiation or biotherapy for this tumor.
- Zubrod 0-1.
- WBC ≥ 4000/mm³ or ANC = 1800/mm³; platelets ≥ 100,000/mm³; total bilirubin ≤ 1.5 mg/dl, creatinine ≤ 1.5 mg/dl or creatinine clearance >50 ml/min, SGOT ≤ 50; PT, PTT < 1.25 x normal (*prior to coumadin*), fibrin split products < 2 x normal, fibrinogen > 200 mg/dl.
- No minor surgery (*e.g. port placement*) less than two weeks prior to study entry; no major surgery less than four weeks prior to study entry.
- No pregnant or lactating women.
- Signed study-specific consent prior to study entry.

Required Sample Size: 46

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ELIGIBILITY CHECK (8/1/01)

Case #

(page 1 of 3)

- _____(Y) 1. Is the malignancy a primary or recurrent (*after surgery only*) soft tissue sarcoma?
- _____(1-2) 2. What is the grade (*using a 4-tiered system*)?
- _____(Y) 3. Was histologic confirmation based on (*ideally*) an open incisional biopsy or several core biopsies of tumor/stromal interface done no more than 2 months prior to registration?
- _____ 4. What is the location of the sarcoma (*upper extremity, lower extremity, body wall*)?
- _____(Y) 5. Is the greatest dimension of the lesion greater than 5 cm?
- _____(Y) 6. Is the patient's Zubrod 0-1?
- _____(Y) 7. Have all required tests been performed within the time frame specified in Section 4.0?
- _____(N) 8. Is there any evidence of metastatic disease?
- _____(N) 9. Are there any contraindications to surgery?
- _____(N) 10. Has the patient received any prior radiation, chemotherapy, or biotherapy for this tumor?
- _____(Y/N) 11. Has the patient had a previous malignancy other than adequately treated non-melanoma skin cancer or cervical cancer *in-situ*?
- _____ (Y) If yes, has the patient been disease free for ≥ 5 years?
- _____(N/NA) 12. Is the patient pregnant, lactating or not using effective contraception? (*code NA for men and for women with non-childbearing potential*)
- _____(N) 13. Does the patient have an active uncontrolled bacterial, viral, or fungal infection?
- _____(N) 14. Does the patient have any serious medical or psychiatric illness that would prevent informed consent or limit survival to less than 2 years?
- _____(N) 15. Has the patient had any CHF or MI within the past six months?
- _____(≥ 4) 16. a. What is the WBC (*per 1000*)?
- _____ (= 1.8) b. What is the ANC (*per 1000*)?
- _____(≥ 150) 17. What is the platelet count (*x 1000*)?
- _____(≤ 1.5) 18. What is the total bilirubin?

(cont'd on next page)

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ELIGIBILITY CHECK (8/1/01)

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Case # _____

- _____(≤ 1.5) 19. What is the creatinine?
- _____(≤ 50) 20. What is the SGOT?
- _____ (Y) 21. PT, PTT < 1.25 times normal (*prior to coumadin*)?
- _____ (Y) 22. Fibrin split products < 2x normal?
- _____ (Y) 23. Fibrinogen > 200 mg/dl?
- _____ (Y) 24. Is it more than two weeks since minor surgery (*e.g. port placement*) or more than 4 weeks since major surgery?
- _____ (N) 25. Does the patient have a history of a bleeding or clotting diathesis?
- _____(N) 26. Does the patient have uncompensated coronary artery disease?
- _____(N) 27. Does the patient have peripheral vascular disease?

The following questions will be asked at Study Registration:

- _____ 1. Name of institutional person registering this case?
- _____(Y) 2. Has the Eligibility Checklist (*above*) been completed?
- _____(Y) 3. Is the patient eligible for this study?
- _____ 4. Date the study-specific Consent Form was signed? (*must be prior to study entry*)
- _____ 5. Patient's Name
- _____ 6. Verifying Physician
- _____ 7. Patient's ID Number
- _____ 8. Date of Birth
- _____ 9. Race
- _____ 10. Social Security Number
- _____ 11. Gender
- _____ 12. Patient's Country of Residence

(cont'd on next page)

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ELIGIBILITY CHECK (8/1/01)

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Case # _____

_____ 13. Zip Code

_____ 14. Patient's Insurance Status

_____ 15. Will any component of the patient's care be given at a military or VA facility?

_____ 16. Treatment Start Date

_____ (Y/N) 17. Is this patient going to receive IMRT?

_____ 18. Tumor Status: 1. Primary or 2. Recurrent

_____ 19. Treatment Assignment

The Eligibility Checklist must be completed in its entirety prior to calling RTOG. The completed, signed, and dated Checklist used at study entry must be retained in the patient's study file and will be evaluated during an institutional NCI/RTOG audit.

Completed by _____

Date _____

1.0 INTRODUCTION

1.1 Sarcomas of Soft Tissue

Soft tissue sarcomas are uncommon malignancies. It is estimated that there are approximately 8100 newly diagnosed cases per year in the United States, which represents 0.66% of the invasive malignant neoplasms reported per year in this country. Approximately 60% of soft tissue sarcomas occur in the lower extremities with the remaining 40% distributed throughout the body. Historically, radical surgical resection has been the treatment of choice for soft tissue sarcomas. Some of the very best results using surgery alone as a single modality treatment have achieved local control rates of approximately 80%. However, these results were often accomplished with the use of surgical amputation in nearly half of the patients in these series.^{1,2} Marginal surgical resection alone with functional limb-sparing intent results in unacceptably high local recurrence rates of 70% or more.³ Through a series of retrospective reports, it was clinically established that limb-sparing surgery (*LSS*) and radiation therapy (*RT*) administered either preoperatively or postoperatively could achieve local control rates of 80% or better in single institutional series.^{4,5,6} A prospective randomized clinical trial evaluating recurrence-free survival in patients treated with either *LSS* and *RT* vs. amputation without *RT* indicated result equivalency.⁷ The tendency in the United States for defining standard of care for management of large high grade or low/intermediate grade soft tissue sarcomas of the extremity with *LSS* and *RT* was further substantiated by a recently published randomized study.⁸ In this study, there was a significant difference in the reported local recurrence rate favoring those patients randomized to combined treatment. The question of whether it is more efficacious to deliver *RT* before or after the surgical resection is subject to controversy, each having advantages as well as disadvantages. In retrospective analysis from single institutions, large soft tissue sarcomas of the proximal lower extremity reportedly have better sustained local control with preoperative radiation therapy.^{9,10} To date however, there is no definitive study that has clearly demonstrated better clinical outcomes for either approach. This preference for treatment sequence appears to remain physician as well as institution dependent.

1.2 Radiation Therapy and Combined Modality Treatment for Soft Tissue Sarcomas

Despite the obvious advantage of combination treatment strategies of surgery and radiation therapy for soft tissue sarcomas, local recurrence rates remain a problem particularly in those patients with large deep extremity sarcomas where margin status is often compromised by the necessity for limb salvage.^{11,12} Additionally, there is some evidence that suggests that the incidence of local recurrence is quite dose dependent and that higher doses of radiation therapy, although demonstrating correlation to improved local tumor control, are often associated with increasing complications and result in limb dysfunction.¹³ Improvements in radiation delivery including hyperfractionation and conformal treatment planning have added to enhanced combined modality efficacy without significantly adding toxicity. However, future improvements in clinical trials outcome with this combined treatment will likely result from a better understanding of radiobiology and the molecular mechanisms of cell response to stress including stress induced by ionizing radiation.¹⁴ Accordingly, there is some early experimental data suggesting that radio-resistance could be mediated by tumor angiogenic response to hypoxic stress induced by the ionizing radiation.¹⁵

1.3 Targeted Therapy Against Pro-Angiogenic Tumor Response

One area of increasing interest in molecular oncology is in the development of new targeted cancer therapeutics that may prove to be most valuable in combination treatment with standard therapy such as *RT*. A focal point within the area of targeted research is centered around therapy against tumor stromal elements particularly those cellular elements responsible for angiogenesis. It has been established that conversion to the angiogenic phenotype is an important event in the evolution of solid tumors and the initiation of the pro-angiogenic switch is necessary for tumor growth and metastatic potential.^{16,17} In recent years, a number of novel compounds have been described that target tumor neovascularity either by direct effect on stromal endothelial cells or by inhibiting signaling pathways that activate endothelial cell migration or expansion.¹⁸ Accordingly, pre-clinical models have suggested that vascular endothelial growth factor (*VEGF*) and its active receptor (*Flk-1/kdr*) play an important role in tumor induced angiogenesis.^{19,20} This has been demonstrated in different studies where the receptor pathways have been disrupted utilizing dominant negative strategies or neutralizing antibodies.^{21,22} The *VEGF* receptor tyrosine kinase inhibitors are a class of drugs that have favorable toxicity profiles, do not induce immune response, and are not susceptible to enzymatic inactivation.²³ These drugs would be ideally suitable for clinical trials. Additionally, there are a number of retrospective studies of human tumor material that have identified *VEGF* expression as an important variable in disease progression and patient survival.^{24,25} In a recent analysis of 80 sarcoma specimens at Fox Chase Cancer Center (*manuscript submitted for publication*), there was a significant correlation between *VEGF* expression within the tumor stroma and overall tumor grade. This data

suggests that "anti-VEGF therapy" may provide a clinical useful option in a treatment scheme directed towards these particular solid tumors.

1.4 SUGEN Compound 5416

SU5416 is a small molecule that exhibits a potent selective inhibition of the Flk-1/kdr receptor tyrosine kinase. Although there are a number of receptors whose expression is restricted to endothelial cells, it is clear that the KDR tyrosine kinase receptor plays a critical role in angiogenesis.²⁶ The mechanism of action of SU5416 appears to be mediated through the VEGF receptor where the compound inhibits the autophosphorylation response to VEGF which subsequently disrupts the intracellular signaling pathways for VEGF mitogenic initiation of endothelial cell proliferation and migration. Phase I studies employing SU5416 as a single agent have demonstrated toxicity profiles with the MTD at 145 mg/m² in a dose schedule of i.v. administration twice per week. The overall toxicity profile of this small molecule compound suggests good bioavailability and quite minimal myelotoxicity making it an ideal drug for combined modality therapy trials.

1.5 Combination Therapy with SU5416

There is compelling pre-clinical data that supports a trial evaluating the use of a targeted angiogenic inhibitor and radiation therapy as combined modality treatment. It has been shown that these two modalities may be synergistic with additive clinical effect and without additive toxicity. A recent publication evaluating both in vitro and in vivo data found that ionizing radiation creates a hypoxic stress response mediated by the endothelial cell compartment of the tumor and manifested by increased VEGF levels. This results in an angiogenic rebound response by the tumor. However, the combined effect of radiation therapy and anti-VEGF therapy mitigates the angiogenic upregulatory response, thereby overcoming inherent radio-resistance and enhancing the overall efficacy of radiation therapy.²⁷ The efficacy of this type of combination therapy in pre-clinical models has also been suggested in several other recent publications.^{28,29,30} Theoretically, the use of this combined therapy targets both the tumor cell compartment and the endothelial cell compartment which should translate into enhanced and prolonged clinical response. Since little is known clinically about the combination of anti-angiogenic agents and radiation therapy, within the design of this study we propose to compare the use of SU5416 for primary operable large soft tissue sarcomas grade 1 or grade 2 (*IB or IIA*) in a combined treatment scheme with radiation therapy and surgery. This will be compared to standard therapy (*radiation therapy and surgery alone*). The rationale for this clinical design evaluates the overall toxicity of the combined SU5416 and radiation therapy in a rapid dose escalation scheme. In many centers for sarcoma treatment in the United States, limb salvage and radiation is the acknowledged standard therapy for large grade 1 and grade 2 soft tissue sarcomas. This study allows for a comparison of an experimental arm to the treatment standard for evaluation of both toxicity and the important biological tissue and blood determinant surrogate endpoints. Although an arm with SU5416 alone could be considered, it was determined that a treatment arm of unproven efficacy such as SU5416 given as a sole modality therapy for 6-8 weeks prior to the surgical management of an operable sarcoma would pose problems in ethical considerations by both patients and institutional review committees. An additional important aspect of this study design will investigate specific described biological endpoints (*see Appendices VII thru XI*) for the purpose of assessing the expression of angiogenic markers both pre- and post-drug administration as a surrogate measure of response to SU5416 and as indirect validation of the proposed molecular target. This data will then be compared to similar analysis in the RT alone arm providing for the assessment of the potential effect of SU5416 on tumor angiogenesis in vivo and whether there is any evidence of response synergism in the combined treatment arm. Relevant correlative tissue studies will be accomplished by collecting blood and tissue specimens at specified intervals within the protocol (*i.e. initial diagnostic biopsy and at the time of surgical resection*) for the purpose of quantitating direct VEGF activity in the tumor cells by immunohistochemistry as well as evaluation of tumor cell and endothelial cell apoptotic and proliferative indices. Additionally, microvessel density (*MVD*), a surrogate of induced angiogenesis, will be determined. Blood specimens will provide for measurement of soluble circulating factors associated with VEGF expression by the sarcoma. These patients with large predominantly extremity malignancies are clearly good candidates for the necessary tissue procurement strategies required by this study.

Although this phase I/II trial is strictly a toxicity and biological end-result study, it is conceivable that result analysis may provide further rationale for combined anti-angiogenic therapy and radiation therapy in future studies for patients with soft tissue sarcomas. Additionally, since the purpose of anti-angiogenic therapy may be angiostasis, the use of biological endpoints rather than the traditional objective response criteria may be a logical way to quantitate response in these patients.³¹

2.0 OBJECTIVES

- 2.1** To assess the dose escalation toxicity of SU5416 and radiation therapy in a clinical design for the purpose of determining the maximum tolerated dose (*MTD*) of SU5416 in a combined modality setting. This will include an assessment of surgical wound healing complications secondary to either radiation alone or to the combined treatment.
- 2.2** To assess whether SU5416 has quantitative anti-angiogenic effects *in vivo*. This will be accomplished by systematic evaluation and comparison of pre- and post-drug biomarker tissue surrogates for microvascular changes. These will include examination of paraffin embedded tumor and tumor/stromal interface for angiogenic regulators (*VEGF, KDR, FIK-1, bFGF*) and tumor/endothelial cell proliferation (*Ki67*) as well as Tunel assay to assess apoptosis in both tumor and endothelial cells. The MVD (*CD34*) will be determined to assess peri-tumoral angiogenesis. In addition, ELISA will be used to quantitate circulating levels of angiogenic growth factors (*VEGF, bFGF*).
- 2.3** To use the above analysis (2.2) to compare tissue specimens and blood from the cohort of patients treated with radiation alone in an attempt to detect evidence of a synergistic anti-angiogenic effect to combined SU5416 and radiation.
- 2.4** To assess disease-free survival, local recurrence, distant metastases, and overall survival rates in patients treated with radiation alone and with radiation combined with SU5416.

3.0 PATIENT SELECTION

3.1 Eligibility Criteria

- 3.1.1** Patients must have a primary or recurrent soft tissue sarcoma confirmed by a pathologist as grade 1 or 2. Biopsy should ideally be an open incisional biopsy (*not a needle biopsy*) or several core biopsies done under CT guidance no more than two months prior to registration.
- 3.1.2** Sarcoma located on the upper (*includes shoulder*) or lower (*includes hip*) extremities or on the body wall.
- 3.1.3** AJCC (1998) Stage IB and IIA (*> 5 cm*) will be included in this study (*see Appendix III*).
- 3.1.3.1** On preoperative chest CT scans, patients may have ≤ 4 lung lesions that are all ≤ 5 mm in diameter each and still be eligible for this protocol providing there is no evidence of lung metastases. They may be $= 1$ cm if demonstrated to be stable over a period of one year and fit the criteria for granulomas. This is done because of the propensity of most patients over 60 to have some parenchymal lesions, which are not pathologic.
- 3.1.4** Zubrod performance status should be 0-1.
- 3.1.5** $WBC \geq 4,000/mm^3$ or $ANC = 1800/mm^3$, platelets $\geq 100,000/mm^3$, bilirubin $\leq 1.5/mg/dl$, creatinine ≤ 1.5 mg/dl or creatinine clearance > 50 ml/min, SGOT ≤ 50 ; PT, PTT < 1.25 times normal (*prior to coumadin*), fibrin split products < 2 x normal, fibrinogen > 200 mg/dl.
- 3.1.6** Treatment must begin within two weeks after registration. Pretreatment evaluations in Section 4.0 must be completed prior to registration.
- 3.1.7** Patients must use effective contraception; must not be pregnant or lactating due to potential exposure of the fetus to RT (*and Sugen 5416*) and unknown effects of RT (*and Sugen 5416*) to lactating females.
- 3.1.8** No evidence of metastases.
- 3.1.9** No contraindications to surgery.
- 3.1.10** Patient must sign a study-specific informed consent form prior to study entry.

3.2 Ineligibility Criteria

- 3.2.1** Prior treatment with radiation, chemotherapy, or biotherapy for this tumor.
- 3.2.2** Minor surgery (*e.g. port placement*) less than two weeks prior to study entry; major surgery less than four weeks prior to study entry.
- 3.2.3** Any sarcoma of the head and neck or intra-abdominal or retroperitoneal sarcoma.
- 3.2.4** Histopathology is desmoid tumor or dermatofibrosarcoma protuberans.
- 3.2.5** No prior or concurrent malignancies other than surgically treated carcinoma *in situ* of the cervix and squamous or basal cell carcinoma of the skin are allowed within the preceding five years.
- 3.2.6** Patients may have no serious medical or psychiatric illness, which would prevent informed consent or limit survival to less than two years.
- 3.2.7** Congestive heart failure or myocardial infarction within previous six months.
- 3.2.8** Patients with uncompensated coronary artery disease.
- 3.2.9** Patients with peripheral vascular disease.
- 3.2.10** Active uncontrolled bacterial, viral or fungal infection until these conditions are corrected or controlled.
- 3.2.11** Severe peripheral vascular disease in any patient, peripheral vascular disease in patients with diabetes mellitus, history of deep venous or arterial thrombosis (*including pulmonary embolism*) within 3 months.

4.0 PRETREATMENT EVALUATIONS

- 4.1** Pre-study blood tests to be done within two weeks prior to registration; imaging studies to be done within four weeks prior to registration.
- 4.1.1** History and physical examination with special attention to measures of primary tumor.
- 4.1.2** PA and lateral CXR.
- 4.1.3** CT scan of chest prior to registration in protocol.
- 4.1.4** CBC, differential and platelet count, PTT, PT (*INR*), fibrinogen, fibrin split products, SGOT, alkaline phosphatase, serum creatinine, serum calcium, total bilirubin.
- 4.1.5** Blood for analysis of circulating VEGF and bFGF levels sent to Fox Chase Cancer Center (*see Appendix X*).
- 4.1.6** EKG, pregnancy test as applicable.
- 4.1.7** Plain films and i.v. enhanced MRI or computerized tomography (*CT*) of involved extremities prior to biopsy will be required at all participating institutions. CT is adequate for tumors of the torso.
- 4.1.7.1** Additional preoperative imaging will be optional and institutional specific. They will be performed for the purpose of assessing whether there is a non-invasive method of detecting quantitative differences in tumor perfusion both pre- and post-SU5416 (*presumed anti-angiogenic effect on tumor microvasculature*). These studies: dynamic MRI and technesium 99m radiolabeled red cell perfusion scintigraphy are described in Section 11.9 and 11.10.

5.0 REGISTRATION PROCEDURES

- 5.1** Patients can be registered only after pretreatment evaluation is completed and eligibility criteria are met. Patients are registered prior to any protocol therapy by calling RTOG headquarters at (215) 574-3191, Monday through Friday, 8:30 a.m. to 5:00 p.m. ET. The patient will be registered to a treatment arm and a case number will be assigned and confirmed by mail. The Eligibility Checklist must be completed in its entirety prior to calling RTOG. The completed, signed, and dated Checklist used at study entry must be retained in the patient's study file and will be evaluated during an institutional NCI/RTOG audit.

6.0 RADIATION THERAPY

6.1 General Guidelines

- 6.1.1** In general, the entire compartment need not be covered. A margin of 5-7 cm is recommended beyond the gross disease in the longitudinal direction. If this causes the field to extend beyond the compartment, the field can be shortened to include the end of the compartment plus a margin of 2 cm.
- 6.1.2** Scars should be bolused with appropriate thickness specific to the energy of the photon beam. A wider area of bolus should be used if there is subcutaneous or cutaneous involvement.
- 6.1.3** Prior biopsy sites should be tattooed so that they can be excised at the time of surgery. This should be done in such a way as to not be confused with the isocenter tattoo.
- 6.1.4** Every effort should be made to:
 - a) Avoid treating the full circumference of an extremity;
 - b) Avoid treating anus, urogenital tract, perineum and genitalia;
 - c) Avoid treating the lung, through use of appropriate shielding and treatment planning;
 - d) Avoid dose maximums in areas where surgical scars will be placed; this requires reviewing treatment plans with the surgeon;
 - e) If possible, avoid treating skin over areas commonly traumatized to full dose (*e.g., the elbow, knee, shin, femoral neck*).

6.2 Preoperative Radiation Therapy

- 6.2.1** Treatment is to consist of 50 Gy in 25 fractions, delivered at 2 Gy per fraction over 5 weeks.
- 6.2.2** The target volume of radiation therapy will include the site of the primary lesion and those tissues suspected of having microscopic disease. In addition to physical exam findings, MRI scans or CT scans obtained during evaluation will be used in defining the target volume. The margins beyond clinically or radiologically evident sarcoma will be 5-7 cm. Optimal field arrangement, beam parameters and shaped blocks will be used to achieve the closest approximation of treatment volume to target volume to minimize irradiation of uninvolved normal tissue.
- 6.2.3** Immobilization devices should be used daily to ensure reproducibility of treatment.

6.3 Post-Operative Radiation Therapy.

- 6.3.1** Postoperative external beam radiation therapy (*EBRT*) boost will be given for patients with positive microscopic or macroscopic margins. The radiation treatment is to be completed by administering 16 Gy to the bed of the residual tumor (*including a margin of 1 cm*). Boost will not be given for patients with 100% necrosis. EBRT will begin approximately 2 weeks following resection, assuming there is satisfactory

healing of the surgical wound. At the time of resection, metallic clips will be placed to aid in defining the tumor bed. The target volume for post-operative radiation therapy will be the tumor bed as defined by the operative and pathological findings.

6.3.2 External Beam Post-Operative Boost Guidelines

- 1) The dose is 16 Gy in 8 fractions (*once a day*).
- 2) Bolus can be avoided unless positive margins occur in cutaneous or subcutaneous tissues.
- 3) It is not necessary to include the entire surgical bed, drain sites and wound.
- 4) Surgical staples should remain in place during the boost.

6.3.3 Three doses of SU5416 should be administered at pre-operative tolerated dose with post-operative radiation therapy for patients with positive microscopic or macroscopic margins.

6.4 Dose Specifications

6.4.1 For two opposed coaxial equally weighted beams: on the central ray at separation of beams.

6.4.2 For an arrangement of two or more intersecting beams: at the intersection of the central ray of the beams.

6.4.3 Any other field arrangement: at the center of the target volume.

6.5 Expected Toxicities

6.5.1 The majority of patients are expected to have Grade 2 skin toxicity or less using the CTC Version 2.0. In the event that confluent, moist desquamation (*Grade 3 toxicity*) is seen involving = 1/3 of the treatment field, this would be unexpected and radiotherapy should be stopped for = 3 days or until evidence of early healing is seen.

6.5.2 Skin Toxicity Grade/Description/Treatment:

Toxicity Grade	Description	Treatment
0	No change over baseline	No treatment required
1	Follicular, faint or dull erythema, epilation/dry desquamation/ decreased sweating	Aquaphor emollient only
2	Tender or bright erythema, patchy moist desquamation/ moderate edema	Lantiseptic cream and/or antibacterial/antifungal cream
3	Confluent, moist desquamation, = 1.5 cm diameter, not confined to skin folds; pitting edema	Vigilon dressing and treatment break = 3 days if = 1/3 treatment field is involved
4	Ulceration, hemorrhage, necrosis	Serious consideration given to discontinuing protocol treatment

7.0 DRUG THERAPY

Institutional participation in chemotherapy studies must be in accordance with the Medical Oncology Quality Control guidelines stated in the RTOG Procedure Manual.

Treatment	Radiotherapy Days	SU5416 and Radiotherapy Days	SU5416 Days After Completion of RT	Surgery Day	Postoperative Radiotherapy ^c
Sugen 5416		2,4,9,11,16,18,23,25,30,32	37,39,44, 46, 51,53,58,60 ^a		84 ^d
RT	1-5, 8-12, 15-19, 22-26, 29-33				84 ^d
Surgery				48-62 ^b	

- Dose number dependent on date of surgery. SU5416 to stop 2 days prior to surgery.
- Performed within this range.
- 2 weeks of radiation (16Gy/8fractions) for positive microscopic or macroscopic margins only.
- To begin 2 weeks after surgery assuming adequate healing; SU5416 concurrently administered twice a week during complete course of post-operative radiotherapy at pre-operative tolerated dose.

7.1 Dose

7.1.1 Sugén 5416 will begin at a dose level of 110 mg/m² for the first 6 patients and then at 145 mg/m² (according to toxicity criteria and study design a 30% dose escalation to 145 mg/m² - See Section 7.1.2). Sugén 5416 will be given as a continuous intravenous infusion over 1 hour via a central or peripheral line twice weekly on Mondays and Thursdays or Tuesdays and Fridays of each week throughout preoperative radiation therapy and after radiation therapy stopping two days prior to surgical resection. Administration may be changed +/- one day to allow for specific administrative reasons (e.g. clinic closure on a Monday national holiday). The first infusion of SU5416 will be administered at 100 cc/hr for the first 15 minutes before increasing to 200 cc/hr. If the patient has evidence of venous irritation or phlebitis (or if the patient cannot tolerate this rate of infusion), the infusion rate may be slowed to 100 cc/hr. Time of drug administration and site of injection is to be recorded. Patients will be observed for at least 3 hours after their first 3 doses. Vital signs will be recorded every 30 minutes after the first 3 doses at which point patients may be observed for only one hour after drug administration. No patients will be started on the 145 mg/m² dose level until the 110 mg/m² dose level has been determined to not be dose-limiting as described in Section 7.1.2. Similarly, no patient will continue at the 145 mg/m² dose level until the 145 mg/m² dose level has been determined to not be dose-limiting as described in Section 7.1.2.

7.1.2 Clinical Design and Dose Modification

Built into the clinical design of this study is a phase I dose escalation or dose reduction schema. Prior phase I studies with SU5416 have indicated that the MTD is 145 mg/m² as a single agent. There are no corresponding clinical studies evaluating the effects of concomitant SU5416 and radiation therapy. The phase I data indicated that a 30% dose escalation scheme was safe and that the only grade 4 toxicity seen in any of the patients on the phase I studies was dose limiting nausea, vomiting, and headaches at 190 mg/m² which resolved with a 30% dose reduction to 145 mg/m². The design of the present study will begin at a dose level of 110 mg/m², which is a 30% reduction from the known MTD dose. This should be a safe initial dose level since there should not be any competitive toxicity with radiation. Dose limiting toxicity (DLT) for the purpose of this study will be defined as grade 3 non-hematologic (including hepatic) and grade 4 neutropenia and thrombocytopenia requiring hospitalization for sepsis syndrome or organ infection that are thought due to SU5416. Toxicities arising pre-surgery during the administration of SU5416 with or without radiation therapy will be used to determine the DLT. Drug is held until resolution of the DLT to baseline or grade 1, at which time it is restarted at the next lowest dose level (*i.e.* 30% reduction in dose: 145 } 110 } 85 } 60 mg/m²; see Section 7.3 for list of specific toxicities). Subsequent cycles will then continue with the reduced dose of SU5416 as tolerated. Any lethal toxicity associated with drug will put the study on hold until formal evaluation takes place.

The first 6 patients will be assigned to the combination therapy arm of the study at 110 mg/m². Doses of SU5416 should not be escalated with individual patients once treatment has been initiated. Toxicity will be evaluated in cohorts of 3 patients. While toxicity is being evaluated, patients will be entered onto the RT only arm. If there is no observed dose limiting toxicity in these first 3 (0/3) patients, then dose will escalate to 145 mg/m² (a 30% increase). If DLT is encountered in one of the first 3 (1/3) patients, then the next 3 patients will be evaluated. If no dose-limiting toxicity is seen in this group of 3 patients (0/3), the dose will escalate. If one or more of this next group of 3 patients experience DLT (1/3), subsequent patients will be treated at 85 mg/m² (which will be the new established MTD). If the dose is escalated to 145 mg/m², this dose will be evaluated in the same manner. Six patients will be treated at this dose level, and toxicity will be evaluated in cohorts of 3 patients. If 0/3 experience DLT, this dose will be considered the MTD. If 1/3 experience DLT, the next 3 will be evaluated. If 0/3 experience DLT in this next group, this dose will be considered the MTD. If 1 or more patients experience DLT in this second group of 3, then the dose will be reduced back to 110 mg/m² and this will be considered the MTD. In addition, if at any time, two patients on a given dose level experience DLT, then the dose will be reduced by 30% and the reduced dose will be considered the MTD for the study. After the MTD for this study has been established, then any DLT on an individual patient basis will require a 30% dose reduction (*see Section 7.3*). RTOG Headquarters will assign the dose determination of SU5416 in the combined modality group when the patient is registered for the study.

7.1.3 Pretreatment Drug Regimen

This pretreatment must be followed for all patients receiving SU5416. To prevent possible hypersensitivity reactions to Cremophor (*one of the excipients in the formulation*) patients are pre-medicated as follows:

H1 blocker diphenhydramine (25-50 mg i.v. or orally 30 to 60 minutes prior to SU5416 injection; or an equivalent dose of an alternate H1 blocker such as loratadine or fexofenadin).

H2 blocker (famotidine 20 mg i.v. or orally 30 to 60 minutes prior to SU5416 injection; or an equivalent dose of an alternate H2 blocker).

Dexamethasone (administered at a dose of 10 mg orally 12 and 6 hours prior to the infusion of SU5416).

In general, the first dose of SU5416 should be administered using a dose of dexamethasone of 10 mg p.o.; subsequent doses of dexamethasone may be reduced to 4 mg 12 and 6 hours prior to injection, if tolerated. If therapy is interrupted, the dexamethasone dose at re-initiation of treatment should be 10 mg. In addition, low dose dexamethasone (0.5-1 mg p.o.) may be administered on the day following SU5416 infusions, if clinically indicated to ameliorate signs of steroid withdrawal.

Patients receiving SU5416 will also receive coumadin 1 mg/day or Dalteparin 2500 IU SQ daily or Lovenox 30 mg SQ beginning on Day 2 and then taken daily to the last day of SU5416 administration as prophylaxis against thromboembolic phenomena (*only during SU5416 administration; to be discontinued within two days of surgery*).

7.1.4 Drug Mixing

SU5416 drug product contains Cremophor®, a solvent that can leach the plasticizer, DEHP, from pliable plastics. SU5416 drug product is therefore not compatible with standard PVC intravenous bags and administration sets. SU5416 is also highly insoluble in aqueous solutions. The drug product must be

added into empty intravenous bags made of polyethylene-lined materials. As empty glass bottles may contain residual amounts of water or saline, they are not compatible with SU5416 drug product. Most intravenous bags made for the delivery of paclitaxel, nitroglycerine, and or fat emulsions are appropriate for the delivery of SU5416. Appropriate bags and administration sets will be used as per RTOG guidelines for the administration of paclitaxel. Examples of suitable i.v. bags include: McGaw HyperFormer E.V.A. mixing container 250 ml or Medstream Vitalmix non-DEHP container 250 ml.

Suitable administration sets are made from low absorption polyethylene tubing or polyethylene lined tubing. SU5416 must be administered through an i.v. administration set which contains a 0.22 micron filter made from hydrophilic polyethylsulphone (*filters made from this material are often described as "extended life" or "hydrophilic" filters*). As excipients in the formulation are capable of dissolving filters made from cellulose acetate, administration sets containing cellulose acetate filters are not compatible with SU5416 drug product. In cases where tubing made from appropriate materials with polyethylene lining can be identified that does not contain an in-line filter, an extension set containing an in-line filter may be added in series.

Examples of suitable i.v. administration sets include:

-Fox Baxter series 8200 Flo-Gard pumps: Administration set 2C1042 (*without in-line filter*) plus an extension set with polysulphone filter.

-For IVAC series 560-570 i.v. pumps: Administration set 1C2053 (*without in-line filter*) plus extension set C20350 with polysulphone filter.

-For IVAC series 580 i.v. pumps: Administration set 1C2053 (*without in-line filter*) plus extension set C20350 with polysulphone filter.

-For GEMINI PC-1 through PC-4 infusion devices: Administration set for fat emulsion and nitroglycerin IM 2260-0500 plus extension set with polysulphone filter. Other administration sets designed to deliver paclitaxel are suitable, provided that the filter is not made from cellulose acetate.

For i.v. administration sets without an in-line filter, suitable extension sets include:

-MedStream extension set MS426 non-PVC 12 inch long extension set with hydrophilic polyethylsulphone filter;

-IVAC extension set C20350;

The actual administration set used will depend upon the type the of i.v. pumps at the site; any set per institutional local practice that fits the criteria above may be used.

Use of peripheral access devices:

SU5416 can be administered through a peripheral access device such as a peripheral i.v., a PICC line or portacath. The line should be checked for patency prior to administration of SU5416 using standard procedures. Any central line should be flushed following the infusion with normal saline, then heparinized saline via slow injection.

7.2 Sugen 5416 (NSC # 696819; IND# 59025)

7.2.1 Dose Formulation: SU5416 may be supplied as one of two possible yellow-orange sterile parenteral formulations:

1. 4.5 mg/ml formulation in 30 cc vials containing 112.5 mg SU5416 in 25 ml of solution.
2. 4.5 mg/ml formulation in 50 cc vials containing 180 mg SU5416 in 40 ml of solution. Other components of the formulation include: polyethylene glycol 400; polyoxyl 35 castor oil (*Cremophor-EL*); benzyl alcohol and dehydrated alcohol. Each vial is intended for single use only.

The molecular formula of SU5416 is $C_{15}H_{14}N_2O$. The formula weight is 238.29. SU5416 is a yellow-orange sterile parenteral formulation.

7.2.1.1 The SU5416 formulation contains the surfactant Cremophor® EL which is also contained in paclitaxel or Taxol®. Cremophor® EL, in the paclitaxel formulation, extracts DEHP from PVC containers and sets. In the nitroglycerine sets that had paclitaxel infusions run through them, the effluent was analyzed by HPLC for leached DEHP plasticizer. The following sets had significant and unacceptable amounts of leached DEHP: Baxter vented nitroglycerine (2C7552S), Baxter vented basic solution (1C8355S), McGAW Horizon pump vented nitroglycerin (V7450), and McGAW intelligent pump vented nitroglycerin (V7150). Although these sets were largely non-PVC, their highly plasticized pumping segments contributed the DEHP. Some earlier study materials distributed by SUGEN indicated that all nitroglycerine sets could be used with SU5416. This is not the case. Please refer to the paclitaxel monograph in "Handbook on Injectable Drugs", 11th Edition, for additional information on specific manufacturer products compatible with paclitaxel infusions.

7.2.2 Preparation: Using routine aseptic procedures, aspirate the correct volume of SU5416 into the syringe. Each vial is intended for single use only. The stoppers in these vials are not designed for multiple punctures. In order to avoid serious drug accountability problems, please do not share partial vials

between patients even though this is tempting if you are preparing more than one patient infusion at a time. Inject solution into an empty non-PVC intravenous bag. As empty glass bottles contain residual amounts of saline or water, they are not compatible with SU5416 drug product. It is very important that the drug is added to an empty bag first and the diluent added afterwards. This is to avoid any chance of precipitation. If the diluent is added first followed by the drug, the mixture will precipitate. Dilute each 1 ml of SU5416 required to prepare the calculated dose with 2 ml of 0.45% Normal Saline; i.e. take 1 part drug product and add two parts of 0.45% normal saline (*sodium chloride*). The pH of the drug product after dilution is approximately 6.6. (*due to lower osmolality, sterile water is preferred for administration in a peripheral vein*). Invert and right the bag until the resulting solution is well mixed (*15-20 times*). SU5416 should be given via a non-PVC pump set with a **non-vented spike** to prevent leaking of the infusate through the venting area. Extension tubing with a 0.2 micron-in-line filter not made from cellulose acetate is required. Suitable tubing includes filters made from polysulphone, such as Baxter 0.22-micron High-Pressure Extended Life Filter or SoloPak 16 or 22 inch extension set with a polysulphone filter in an acrylic cassette. Allowance should be made for priming volume of the administration set and the extension set used. The pharmacist should clearly label the i.v. bag with the correct volume of infusate to be infused, study drug name, patient identifier, and the date and time of dilution. When using a peripheral access device (*such as a Mediport or PICC line*), remove the i.v. bag and administration line when the correct volume has been infused and flush only from the catheter port. Do not flush the bag and administration set with saline as this may cause precipitation of SU5416. While SU5416 is considered an anti-cancer agent, there is no evidence to date that it is cytotoxic in the dose range to be studied. It is recommended, however, that precautions to ensure the safe handling of this compound (*particularly the use of gloves*) should be observed by all study personnel. For clinical sites using infusion pumps with a roller clamp mechanism tubing contact with the roller clamp area, there have been reports of some yellow-orange discoloration of the roller clamp area. This is easily removed with an alcohol wipe or 2% bleach solution.

7.2.3 *Mechanism of Action:* SU5416 is a small organic molecule that is a potent and selective inhibitor of the activity of Flk-1, a receptor tyrosine kinase expressed on the surface of the endothelial cell. Binding of vascular endothelial growth factor (*VEGF*) to the extra-cellular domain of Flk-1 activates the tyrosine-kinase activity in the cytoplasmic portion of the molecule, beginning a signaling cascade leading to cellular proliferation. It is believed that the inhibition of this signaling pathway by SU5416 will result in inhibition of endothelial cell proliferation and the sprouting of new vessels in tumors. Because radiation can induce VEGF production in tumors, blockade of the effects of VEGF production with anti-angiogenesis agents like SU5416 produces synergistic anti-tumor effects when combined with radiation. The alpha half-life or distribution phase of the drug is rapid, with a mean value of 10.7 + 4.5 minutes. The beta half-life or elimination phase has a mean value of 87.3 + 35.6 minutes, with a range from 35-235 minutes.

7.2.4 *Supply:* Drug will be supplied by Sugen Pharmaceuticals to NCI for distribution. The Principal Investigator (*or authorized designee*) at each participating institution may request SU5416, from NCI's Pharmaceutical Management Branch (*PMB*). PMB policy requires that drug be shipped directly to the institution where the patient is to be treated. PMB does not permit the transfer of agents between institutions unless prior approval from PMB is obtained. Completed Clinical Drug Requests (*NIH-986*) should be submitted to the PMB by fax (*301*) 480-4612 or mailed to the Pharmaceutical Management Branch, NCI, Executive Plaza North, MSC 7422, Room 7149, Bethesda, MD 20892. The Investigator, or a responsible party designated by the investigator, must maintain a careful record of the inventory and disposition of all drugs received from DCTD, using the NCI Drug Accountability Record Form (*see the NCI Investigators Handbook for Procedures for Drug Accountability and Storage*). If you have additional questions regarding SU5416, contact Mr. Carmen DeBellis, R.Ph., at PMB at 301-496-5725.

7.2.5 *Storage:* Vials of SU5416 must be stored at controlled room temperature 15-30° C (*59-86° F*) and protected from light. Keeping the vials stored in the vial cartons that they arrive in will insure protection from light.

7.2.6 *Stability:* Diluted drug product is stable for 8 hours at room temperature.

7.2.7 *Side Effects:* Side effects: The dose limiting toxicity for SU5416 is nausea, headache and vomiting. *Neurological:* headache (*can often be ameliorated with ibuprofen and acetaminophen, but may require prophylaxis and/or treatment using sumatriptan, a selective serotonin agonist, or narcotic analgesics*). *Vascular:* thromboembolic events including superficial thrombophlebitis, deep vein thrombosis, myocardial infarction, pulmonary embolus, subdural or subphrenic and subcapsular hematoma and cerebral bleeding have been reported although these occurred in patients with underlying cancer and/or cardiovascular disease, so it is not clear that these are drug-induced.

Respiratory: dyspnea, cough, pharyngitis, voice alterations, pneumonia, hemoptysis. All patients in whom dyspnea was severe had either lung cancer or pre-existing respiratory ailments.

Gastrointestinal: nausea and vomiting (*ameliorated by antiemetics*), diarrhea, abdominal pain, anorexia, constipation, hemorrhage.

Hepatic: Elevation of transaminases, alkaline phosphatase, total bilirubin, a recent report of hepatic rupture possible secondary to 5416 in a patient with metastatic lung cancer, or possible hepato-renal insufficiency as reported in a patient with a history of Kaposi's sarcoma.

Metabolic: hypercalcemia; patient should have weekly serum calcium levels while receiving SU5416.

Musculoskeletal: Injection site pain.

Toxicity due to excipients in the formulation such as Cremophor® or absolute alcohol was observed. Cremophor® is similar to that used in paclitaxel (*Taxol*®). Mild to moderate hypersensitivity reactions consisting of flushing, chest pain, dyspnea, and tachycardia have been reported to occur in 41% of patients treated with paclitaxel. Anaphylaxis and severe hypersensitivity reactions have been reported in 2% of patients receiving paclitaxel in clinical trials. Patients receiving SU5416 are thus at risk for anaphylaxis and hypersensitivity reactions. Two patients to date have experienced an anaphylactoid reaction with SU5416 which were reversed with diphenhydramine and dexamethasone. Other hypersensitivity reactions possibly attributable to SU 5416 drug product include fever (28%), dyspnea (26%), vasodilatation, rash (*both 11%*), hypotension (7%), pruritis (6%), and tachycardia (5%). SU5416 should not be given to patients with a history of allergic reactions to paclitaxel. All patients should be observed for one hour after the initial, second, and third infusion. The formulation for SU5416 also contains approximately 22% absolute ethanol. At a dose of 145 mg/m² (*assuming a BSA of 1.8 m²*), the patient will receive approximately 12.7 ml of ethanol over 60 minutes (*equivalent to a glass of beer or wine*) which may produce clinical signs in some patients.

Adverse events thought to be related to the pre-medication regimen (*such as corticosteroid withdrawal or the short-acting toxicities of an antihistamine*) include asthenia, arthralgia, dizziness/vertigo, peripheral edema (17%), increased sweating (11%), myalgia, hypokalemia, pruritis, emotional lability, leukocytosis, abnormal vision, and amblyopia (*all less than 10%*).

No specific toxicity arising from antiangiogenic effects were noted. No effects on menstruation or fertility were observed. Patients with uncompensated coronary artery disease, or with a history of myocardial infarction or severe angina, or peripheral vascular disease are excluded from trials using SU5416. Female patients will be advised on the possible risks prior to study entry. Except for sporadic reporting of vaginal bleeding (*n=2*) or dysmenorrhea (*n=1*), no changes in menstruation have been reported to date. The effects of SU5416 on the developing embryo or fetus are unknown. All patients must be practicing effective birth control while on study and all female patients at risk must have a negative serum pregnancy test prior to receiving drug therapy on this study.

Potential adverse events include hepatocellular damage and/or liver dysfunctions, including one recently non-fatal case of hepatic rupture perhaps secondary to SU5416 complicated by multiple other drugs and metastatic lung cancer. Adverse events also include nausea, vomiting, diarrhea, change in stool and abdominal pain. In males there may be signs and symptoms relating to antiandrogen effect (*such as muscle loss, weight loss, decreased libido, decreased sperm count and decreased fertility*). Damage to the kidneys (*proximal tubules*), Kupffer cells in the liver, spleen and lymphoid tissues may occur following administration of the vehicle polyoxyethylated castor oil or Cremophor. Anaphylactic and severe hypersensitivity reactions attributable to this vehicle have been associated with dyspnea, hypotension, angioedema and generalized urticaria.

7.3 Drug Dose Modification

Dose modifications of SU5416 should be made only for toxicities thought to be due to the SU5416 alone (*i.e.* not expected or related to the radiation alone – refer to Section 6.5).

7.3.1 Gastrointestinal Toxicity:

Nausea and/or vomiting should be controlled with adequate antiemetics. For grade 3 toxicity, reduce SU5416 by 30%. If toxicity recurs, reduce 30% again. If toxicity recurs at this dose level, then discontinue the drug. If Grade 4 nausea/vomiting occurs in spite of antiemetics, the dose should be held until resolution of the DLT to baseline or grade 1, at which time it is restarted at the next lowest dose level, 30%. If toxicity recurs, reduce 30% again. If toxicity recurs at this dose level, then discontinue the drug.

7.3.2 Hepatic Toxicity: give the following percent of previous course's dose based on the patient's bilirubin on the day of treatment.

Bilirubin (mg/dl)	SU5416
> 3.0 - < 5.0	65%
5.0	0%

- 7.3.3** Neurotoxicity:
Headaches: Intractable headaches not controlled by acetaminophen or ibuprofen may be due to SU5416. Patients who develop = grade 3 headache may be retreated with prophylaxis and/or treatment using sumatriptan, a selective serotonin agonist, or narcotic analgesics. Recurrence of = grade 3 headaches despite adequate prophylaxis should be a criterion for dose limiting toxicity; discontinue SU5416 that day and resume at 30% dose reduction. If the same symptoms recur at the reduced dose, discontinue the drug and notify RTOG headquarters and IDB per protocol.
- 7.3.4** Cardiovascular Toxicity:
Thromboembolic events: Treatment and prophylaxis using appropriate anticoagulation should be initiated, but SU5416 should be discontinued only when a thrombotic event, such as a DVT, is accompanied by a symptomatic embolic event such as a pulmonary embolism. RTOG headquarters and IDB should be notified per protocol for the occurrence of any thromboembolic event.
- 7.3.5** Hypersensitivity reactions: All patients experiencing \geq grade 3 hypersensitivity reactions thought to be due to SU5416 will be reported to IDB and RTOG headquarters as an adverse event. All patients experiencing grade 4 hypersensitivity reactions will be removed from the study. If a patient experiences a grade 3 hypersensitivity reaction at a reduced dexamethasone dose (*see Section 7.1.3*), the patient may receive SU5416 with full dose dexamethasone pre-medication. If a patient experiences a grade 3 hypersensitivity reaction with full dose dexamethasone pre-medication, they will be removed from the study and reported to IDB RTOG headquarters.
- 7.3.6** Respiratory: The main cause of shortness of breath may be related to hypersensitivity reactions (*see Section 7.3.5*). To date, SU5416 has not been associated with specific pulmonary toxicity.
- 7.3.7** Metabolic: Reduce SU5416 by 30% for calcium values > 12.5 mg/dl.
- 7.3.8** Miscellaneous Toxicity
Extravasation outside a vein will cause skin necrosis; stop the infusion immediately if extravasation if suspected.
- 7.4** Disease Progression
Patients who develop systemic metastatic disease will be considered treatment failures and will be removed from the protocol treatment but follow-up data will still be collected. They may be treated with other forms of palliative chemotherapy.
- 7.5** Adverse Drug Reaction Reporting
- 7.5.1** This study will utilize the CTC version 2.0 for toxicity and Adverse Event reporting. A copy of the CTC version can be downloaded from the CTEP home page (<http://ctep.info.nih.gov>). All appropriate treatment areas should have access to a copy of the CTC version 2.0. See Appendix V for protocol reporting guidelines.
- 7.5.2** This study will be monitored by the Clinical Data Update System (CDUS) version 1.0. Complete cumulative CDUS data will be submitted quarterly to CTEP by electronic means. Reports are due January 31, April 30, July 31 and October 31.
- 7.5.3** Within 24 hours of discovery, the AE should be telephoned to RTOG HQ DM and to the study chairman. The report should be sent to RTOG within 10 working days. Any death, regardless of cause, which occurs during protocol treatment must be reported to RTOG Headquarters by telephone within 48 hours of discovery.
- 7.5.4** Reporting
- 7.5.4.1** Expedited Reporting for Phase I Studies (including hospitalization defined in bullet 1 below):

UNEXPECTED EVENTS		EXPECTED EVENTS	
Grades 2 – 3 Attribution Possible, Probable, or Definite	Grades 4 and 5 Regardless of Attribution	Grades 1 - 3	Grades 4 and 5 Regardless of Attribution
<p>Grade 2 - Expedited report within 10 working days.</p> <p>Grade 3 - Report by phone to IDB within 24 hours. Expedited report to follow within 10 working days.</p> <p><i>(Grade 1 - Adverse Event Expedited Reporting NOT required.)</i></p>	<p>Report by phone to IDB within 24 hours. Expedited report to follow within 10 working days.</p> <p>This includes deaths within 30 days of the last dose of treatment with an investigational agent.</p>	<p>Adverse Event Expedited Reporting NOT required.</p>	<p>Report by phone to IDB within 24 hours. Expedited report to follow within 10 working days.</p> <p>This includes deaths within 30 days of the last dose of treatment with an investigational agent.</p>
Note 1	Telephone number available 24 hours daily: (301) 230-2330 (Recorder after hours).		
Note 2	See the DCTD/NCI Common Toxicity Criteria		
Note 3	Report to: <i>Investigational Drug Branch, Post Office Box 30012, Bethesda, Maryland 20824. Also report to RTOG Headquarters.</i>		
Note 4	A list of agent specific expected adverse events can be found in the protocol document or consent form.		
Note 5	Reactions judged <i>definitely</i> not treatment-related should not be reported. However, a report should be submitted if there is reasonable suspicion of drug effect.		

- For **Hospitalization** only – Any medical event equivalent to CTC Grade 3, 4, 5 which precipitated hospitalization (*or prolongation of existing hospitalization*) must be reported regardless of expected or unexpected and attribution.
- Expedited reports are to be submitted using AdEERS or the paper templates available at <http://ctep.info.nih.gov>. The NCI Guidelines for expedited adverse reporting are also available at this site.

7.5.4.2 Expedited Reporting for Phase II Studies (*including hospitalization defined in bullet 1 below*):

UNEXPECTED EVENTS		EXPECTED EVENTS	
Grades 2 – 3 Attribution Possible, Probable, or Definite	Grades 4 and 5 Regardless of Attribution	Grades 1 - 3	Grades 4 and 5 Regardless of Attribution
Expedited report within 10 working days. (Grade 1 - Adverse Event Expedited Reporting NOT required.)	Report by phone to IDB within 24 hours. Expedited report to follow within 10 working days.	Adverse Event Expedited Reporting NOT required.	Expedited report, including Grade 5 Aplasia in leukemia patients, within 10 working days. Grade 4 Myelosuppression is not to be reported, but should be submitted as part of the study results. Other Grade 4 events that do not require expedited reporting would be specified in the protocol.
Note 1	Telephone number available 24 hours daily: (301) 230-2330 (Recorder after hours).		
Note 2	See the DCTD/NCI Common Toxicity Criteria		
Note 3	Report to: <i>Investigational Drug Branch, Post Office Box 30012, Bethesda, Maryland 20824. Also report to RTOG Headquarters.</i>		
Note 4	A list of agent specific expected adverse events can be found in the protocol document or consent form.		
Note 5	Reactions judged <i>definitely</i> not treatment-related should not be reported. However, a report should be submitted if there is reasonable suspicion of drug effect.		

- For **Hospitalization** only – Any medical event equivalent to CTC Grade 3, 4, 5 which precipitated hospitalization (*or prolongation of existing hospitalization*) must be reported regardless of expected or unexpected and attribution.
- Expedited reports are to be submitted using AdEERS or the paper templates available at <http://ctep.info.nih.gov>. The NCI Guidelines for expedited adverse reporting are also available at this site.

7.6 Clinical Trials Agreement

The agent(s) (*hereinafter referred to as “Agent[s]”*), used in this protocol is/are provided to the NCI under a Clinical Trials Agreement (CTA) or a Cooperative Research and Development Agreement (CRADA) between Company (*or Companies*) (*hereinafter referred to as “Collaborator(s)”*) and the NCI Division of Cancer Treatment, Diagnosis. Therefore, the following obligations/guidelines apply to the use of the Agent(s) in this study:

Agent(s) may not be used outside the scope of this protocol, nor can Agent(s) be transferred or licensed to any party not participating in the clinical study. Collaborator(s) data for Agent(s) are confidential and proprietary to Collaborator(s) and should be maintained as such by the investigators.

For a clinical protocol where there is an investigational Agent used in combination with (*an*) other investigational Agent(s), each the subject of different CTAs or CRADAs, the access to and use of data by each Collaborator shall be as follows (*data pertaining to such combination use shall hereinafter be referred to as “Multi-Party Data.”*):

- NCI must provide all Collaborators with written notice regarding the existence and nature of any agreements governing their collaboration with NIH, the design of the proposed combination protocol, and the existence of any obligations which would tend to restrict NCI’s participation in the proposed combination protocol.
- Each Collaborator shall agree to permit use of the Multi-Party Data from the clinical trial by any other Collaborator to the extent necessary to allow said other Collaborator to develop, obtain regulatory approval or commercialize its own investigational Agent.
- Any Collaborator having the right to use the Multi-Party Data from these trials must agree in writing prior to the commencement of the trials that it will use the Multi-Party Data solely for development, regulatory approval, and commercialization of its own investigational Agent.
- The NCI encourages investigators to make data from clinical trials fully available to Collaborator(s) for review at the appropriate time (*see #e*). Clinical trial data developed under a CTA or CRADA will be made available exclusively to Collaborator(s), the NCI, and the FDA, as appropriate.

- e) When a Collaborator wishes to initiate a data request, the request should first be sent the NCI, who will then notify the appropriate investigators (*Group Chair for cooperative group studies, or PI for other studies*) of Collaborator's wish to contact them.
- f) Any data provided to Collaborator(s) must be in accordance with the guidelines and policies of the responsible Data Monitoring Committee (*DMC*), if there is a DMC for this clinical trial.
- g) Any manuscripts reporting the results of this clinical trial should be provided to CTEP for immediate delivery to Collaborator(s) for advisory review and comment prior to submission for publication. Collaborator(s) will have 30 days from the date of receipt for review. An additional 30 days may be requested in order to ensure that confidential and proprietary data, in addition to Collaborator(s)'s intellectual property rights, are protected. Copies of abstracts should be provided to Collaborator(s) for courtesy review following submission, but prior to presentation at the meeting or publication in the proceedings. Copies of any manuscript and/or abstract should be sent to:

Regulatory Affairs Branch, CTEP, DCTD, NCI
 Executive Plaza North, Room 718
 Bethesda, Maryland 20892
 FAX (301) 402-1584

The Regulatory Affairs Branch will then distribute them to Collaborator(s).

8.0 SURGERY

8.1 Initial Biopsy

- 8.1.1** Biopsy should ideally be an open incisional biopsy or can be a multiple core biopsy under CT guidance to provide tumor/stromal interface. This is a requirement even if the patient had a prior diagnostic needle biopsy before being evaluated by the participating RTOG institution. The rationale for the preference of open incisional biopsy is that in order for the biologic correlates of the study to be analyzed, it is critical to obtain adequate tissue particularly from the tumor stromal interface. Sufficient tumor must be obtained to determine the diagnosis of soft tissue sarcoma and the tumor grade. The biopsy should be done in such a way as to permit the excision of the biopsy site at the time of formal resection. If the patient had an initial needle biopsy before being evaluated by the particular RTOG institution, then the needle biopsy site should be tattooed for radiation port identification.

8.2 Surgery

- 8.2.1** The surgeon and radiation oncologist will consult after diagnosis and prior to the institution of preoperative therapy. Following preoperative therapy the surgeon, radiation therapist and medical oncologist see the patient again. Definitive plans for resection are made at this time. If deemed necessary, plastic surgery may be consulted at this time.
- 8.2.2** Resection of the sarcoma will occur following combined preoperative radiation and Sugem 5416 or following the radiation alone arm of the study. The resection should be done with a goal to have negative margins. Quality assurance for surgical resection will be provided by assessment of the specimen by surgical pathology (*see Section 10.0*). Absence of tumor on ink will be accepted as a negative margin.
- 8.2.3** Definitions of operative procedures will be made following pathologic evaluation of the resected specimen (*no more than two weeks post operation*). The definitions include:
- 8.2.3.1** Amputations
 - 8.2.3.2** Non-amputation and the margins achieved
 - 8.2.3.2.1** Intralesional Resection - grossly positive margin - visible tumor left behind.
This procedure is not acceptable as a biopsy or a therapeutic resection for the purposes of this protocol.
 - 8.2.3.2.2** Marginal Resection - All gross disease removed; less than compartmental or muscle group excisions; microscopically positive margins. These patients will receive postoperative radiation and continue on protocol (*see Section 6.3*).
 - 8.2.3.2.3** Wide Excision - Microscopically negative margins, less than compartmental or muscle group excision (*for lesion within a specific muscle group*), all gross disease removed. Margins are microscopically negative.
 - 8.2.3.2.4** Radical Excision - Entire anatomic compartment and negative microscopic margin.
 - 8.2.3.2.5** Periosteum - If periosteum is resected in extremity sarcomas, consideration should be given for internal fixation.

8.3 Definitive Surgical Procedure.

The surgical treatment necessary to resect the tumor with negative margins should be used. These definitions noted above will be recorded in the surgical form.

8.4 Principles of Surgery

- 8.4.1** All lesions of the trunk and extremities will be treated with conservative resection (*minimal wide excision*) after preoperative therapy. Any biopsy site should be excised *en bloc* with the definitive surgical specimen. Surgical resection should remove as wide a margin of tissue around the tumor as possible without compromising function. Dissection should always be done through grossly normal tissue planes and should be done beyond the fascial plane adjacent to the tumor. If the tumor is close to or displaces major vessels or nerves, these need not be removed if the adventitia or perineurium is removed and the margin is not involved pathologically. Frozen section at the time of surgery should be done from the closest margin and should be confirmed as being free of tumor. If postoperative pathology evaluation reveals positive soft tissue margins other than bone, nerve or large blood vessels, this margin should be resected if possible. If bone, major blood vessel or nerve is microscopically positive, additional radiation should be given as noted in the protocol. In general, lymph node dissection is not recommended but a sampling can be performed if regional lymph nodes are clinically enlarged or if the primary tumor is over a major node station. Elective node sampling may be performed in patients with clinically positive lymph nodes. Primary tumors overlying major lymph node stations may best be treated with surgical resection including node dissection. Marker clips (*titanium*) should be placed to help guide the radiation oncologist. Closed wound suction drainage should be used in all anatomic regions (*Daval, Hemovac, etc.*). The drains should exit the skin close to the edge of the surgical incision. External compression for extremity resections with ace wraps or compression dressings is advised.
- 8.4.2** State *clearly* in the operative note what type of surgical procedure was performed, and from where the frozen section of the margins was taken.
- 8.4.3** Because all patients will have had radiation special care must be given to skin flaps. Use of muscle flaps, pedicled myocutaneous flaps, and even free flaps is encouraged to fill dead space and used if there is any concern about the viability of the wound flaps.
- 8.4.4** In general, the following principles should be followed in postoperative management of these patients: Maintain staples or skin sutures per surgeon preference, but because of potential delay in wound healing advise 3-4 weeks.
Leave drains until the drainage meets the criteria for surgeon preference for discontinuation.
Begin rehabilitation slowly.
- 8.4.5** Resectability will depend upon the judgment of the operating surgeon. For the extremities, resection must be the limb salvage procedure. For other anatomic areas, it must be judgment of the operating surgeon that he/she may reasonably expect to obtain negative margins. Extremity patients who are not resectable without amputation may be amputated. Unresectable tumors elsewhere may be palliated with additional chemotherapy or radiation therapy.
- 8.5 Protocol Compliance (See also Section 11.2.2)**
- 8.5.1** *Per Protocol* - Surgery completed by day 70 after start of preoperative treatment.
- 8.5.2** *Minor Variation* - Surgery completed between days 70-79 after start of preoperative treatment.
- 8.5.3** *Major Deviation* - Surgery completed \geq day 80 after start of preoperative treatment.

9.0 OTHER THERAPY

Not applicable to this study.

10.0 PATHOLOGY

10.1 Assessment of Pre-treatment Biopsy Specimens

10.1.1 Central Pathology Review (Upon Study Entry)

- a) Recuts of all histology slides, the surgical pathology report, and a representative paraffin block and a Pathology Submission Form should be submitted to:

**LDS Hospital
Dept. of Pathology
E.M. Laboratory
8th Ave & C Street
Salt Lake City, UT 84143
(801) 408-5626
FAX (801) 408-5020
Ldafurne@ihc.com**

10.1.2 Type of Specimen

Note whether the specimen represents:

- a) Incisional biopsy;
- b) Resection Specimen

10.1.3 Histopathological Assessment

- a) Sarcoma phenotype as categorized by the WHO (1994)³²
- b) Histologic grade (*grade 1 or 2*). Grading of soft tissue sarcomas is an imperfect endeavor, not without limitations and pitfalls. This stems from the markedly different histologies between different sarcoma types. All tumors in this study must be either low or intermediate grade (*1 or 2 using a 4-tiered system, 1 or 2 if using a 3-tiered system*). Features such as mitotic rate, cellularity, necrosis, pleomorphism, and differentiation may separate grade. The 3 scale grading system is widely used and should be readily adaptable to this study.
- c) Mitotic rate (*> 6 per HPF*):Yes/No
- d) Necrosis (*0, < 50%, or ≈ 50%*)
- e) Tumor matrix (*sparse, myxoid, fibrous, etc.*)
- f) Vascular space invasion (*Yes/No*)
- g) Host lymphoplasmacytic response (+/-)
- h) Margin of infiltration (*pushing, infiltrative, not evaluable*)

10.2 Assessment of Resected Tumor

10.2.1 Central Pathology Review (Upon Study Completion)

- a) Recuts of all histology slides, the surgical pathology report, and a representative paraffin block and a Pathology Submission Form should be submitted to LDS Hospital per Section 10.1.1.

10.2.2 Gross Parameters of Tumor

- a) Tumor size (*cm greatest dimension*).
- b) Description of margins including cm or mm to closest margin.
- c) Gross photograph of tumor desirable.

10.2.3 Handling of Gross Specimen

- a) External surface specimen should be painted with India ink prior to sectioning.
- b) Tumor should be thoroughly sampled (*at least 1 section per 1 cm of greatest tumor dimension*).

10.2.4 Histopathological Assessment³³

- a) Percent of viable neoplasm (*0, < 25%, 25-50%, > 50-75%, > 75%*)
- b) Percent necrosis (*0, < 50%, > 50%*)
- c) Degree of fibrosis/hyalinization (*0, < 50%, ≈ 50%*)
- d) Tumor margin (*pushing, infiltrative*)
- e) Host lymphoplasmacytic response (+/-)
- f) Vascular space invasion (*Yes/No*)
- g) Surgical resection margin (*+, close, wide*)
- h) Degree of intratumoral hemorrhage (*0, < 50%, ≈ 50%*)

10.3 RTOG Tissue Bank (See Appendices VII – XI for Description and Handling of Specimens)

10.3.1 Biologic studies will be performed both pre-treatment and post-treatment on the paraffin sections including:

10.3.1.1 Immunohistochemistry for angiogenic markers (*CD34, VEGF, bFGF, Flt-1, KDR, FGFR*) and proliferation markers (*Ki-67*).

10.3.1.2 Apoptosis will be assessed by means of the terminal-dioxynucleotidyl transferase-mediated dUTP nick-end labeling (*TUNEL*) assay.

10.3.1.3 Serum/plasma VEGF levels and bFGF levels will be determined at four or five time points during the course of treatment: (1) pre-treatment (*baseline*) and within one hour of completion of first SU5416 infusion; (2) on treatment/pre-resection (*1-2 weeks prior to resection after having received at least four doses of SU5416*) two separate samples: prior to receiving regular scheduled SU5416 dose and within one hour of completion of SU5416 infusion; (3) at resection (*day prior to or morning of surgery – should be obtained prior to surgical incision*); (4) initial post-resection (*3-6 weeks following resection at initial follow-up; see Appendix X*). Patients with positive margins will have one additional serum/plasma VEGF and bFGF level determined 34 months following completion of postoperative chemotherapy and radiation therapy.

10.3.1.4 The biological endpoint analysis of this study will require paraffin blocks from the stromal tumor interface as well as peripheral blood specimens. These tissues will be used to quantitate pre- and post-treatment specimens for the presence of intrinsic markers of angiogenesis in tumor and endothelial cells as well as indirect evidence of anti-angiogenic effect on apoptosis and proliferation of these cells.

In addition, circulating levels of angiogenic factors (*both serum and plasma*) will be analyzed pre- and post-treatment. For detailed descriptions of these four projects see Appendix VII-XI. Tissue samples may be made available to Sugen, Inc. for further analysis.

10.3.1.5 RTOG will reimburse pathologists from submitting institutions \$100.00 per case if proper materials are submitted according to the study translational research protocols (*see Appendix VII-XI*)(*Reimbursement is handled through an invoice submitted to RTOG Administration, ATTN: Path Reimbursement*).

10.3.1.6 Patient consent form should give the Pathology Department authority and responsibility to comply with this request (*pathology blocks belong to the patient from whom tissue has been removed*).

10.3.1.7 Translational research data forms should accompany specimen submitted to LDS Hospital per Section 10.1.1.

11.0 PATIENT ASSESSMENTS

11.1 Study Parameters

Study Parameters	Prior to Study Entry	Every Week During RT/ Sugen 5416	Prior to Surgery	Post RX 6 Weeks	F/U Every 3 Months to Year 2	F/U Every 6 Months Years 2-5	F/U >5 Years Yearly
History and PE	X	X	X	X	X	X	X
Hgt (cm)	X						
Wgt (kg)	X	X	X	X	X	X	X
Body Surface (m ²)	X						
KPS	X		X	X	X	X	X
Biopsy (<i>incisional or multiple core biopsies</i>)	X						
CT of Chest	X ^b		X	X		X ^f	X
EKG	X ^b						
Plain films, MRI or CT of Primary Tumor ^c	X ^b		X	X		X ^f	X
Dynamic MRI ^e	X		X				
SPECT Scan ^e	X		X				
Chest X-ray	X ^b				X		X
VEGF, bFGF (<i>serum and plasma</i>)	X ^a	X ^g	X	X	X ^h		
CBC, Differential, Platelets	X ^a	X	X				
Creatinine, SGOT	X ^a	X	X				
Alkaline Phosphatase	X ^a	X	X	X			
Serum Calcium	X ^a	X					
PT,PTT, fibrinogen, fibrin split products	X ^a	X	X				
Total Bilirubin	X ^a	X	X	X			
Urinalysis	X ^a						
Pregnancy Test ^d	X ^a						

- Two weeks prior to study entry.
- Four weeks prior to study entry.
- MRI should be done in all extremity sarcomas, though CT will be accepted. CT should be done in sarcomas of the torso wall. See Section 11.9 for MRI and optional dynamic MRI guidelines.
- To be performed in all females with childbearing potential.
- Optional, institution specific.
- Do every 6 mos following treatment.
- Blood draw within one hour of first SU5416 infusion; 1-2 weeks prior to surgery, draw two samples: prior to receiving scheduled SU5416 dose and within one hour of completion of SU5416 infusion. (*See Appendix X*).
- VEGF and bFGF in serum and plasma measured at initial 3 month F/U ONLY; patients with positive surgical margins will have one additional measurement 3-4 months following completion of post-operative treatment.

11.2 Response Assessment

11.2.1 Measurement of Response

Response will be evaluated in this study using both the new international criteria proposed by the Response Evaluation Criteria in Solid Tumors (*RECIST*) Committee [JNCI 92(3):205-216, 2000] and the response criteria utilized in the previous high-risk sarcoma study, RTOG 95-14, based upon measurement of perpendicular dimensions (*see Section 11.2.2.2*). Changes in only the largest diameter (*unidimensional measurement*) of the tumor lesions are used in the RECIST criteria. **Note:** Lesions are either measurable or non-measurable using the criteria provided below. The term “evaluable” in reference to measurability will not be used because it does not provide additional meaning or accuracy.

Measurable Disease: Measurable lesions are defined as those that can be accurately measured in at least one dimension (*longest diameter to be recorded*) as ≥ 20 mm with conventional techniques (*CT, MRI, x-ray*) or as ≥ 10 mm with spiral CT scan. All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters).

Target Lesions: All measurable lesions up to a maximum of five lesions per organ and 10 lesions in total representative of all involved organs should be identified as **target lesions** and recorded and measured at baseline. Target lesions should be selected on the basis of their size (*lesions with the longest diameter*) and their suitability for accurate repeated measurements (*either by imaging techniques or clinically*). A sum of the longest diameter (*LD*) for all target lesions will be calculated and reported as the baseline sum LD. The baseline sum LD will be used as reference by which to characterize the objective tumor response.

11.2.1.1 Guidelines for Evaluation of Measurable Disease

All measurements should be taken and recorded in metric notation using a ruler or calipers. All baseline evaluations should be performed as closely as possible to the beginning of treatment. The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up. Imaging-based evaluation is preferred to evaluation by clinical examination when both methods have been used to assess the anti-tumor effect of a treatment.

Clinical lesions: Clinical lesions will only be considered measurable when they are superficial (*e.g., skin nodules and palpable lymph nodes*). In the case of skin lesions, documentation by color photography, including a ruler to estimate the size of the lesion, is recommended.

Conventional CT and MRI: These techniques should be performed with cuts of 10 mm or less in slice thickness contiguously. Spiral CT should be performed using a 5 mm contiguous reconstruction algorithm. This applies to tumors of the chest, abdomen, and pelvis. Response to pre-operative chemotherapy and radiation will be measured by comparing tumor size at time of registration with measurements taken 0-2 weeks prior to surgical resection. Response will be measured through evaluation of the MRI change, CT change, or change in physical examination. In addition, pathologic response will be recorded at the time of pathologic review of the resected specimen (*see pathology section*).

11.2.2 Response Criteria

Response and progression to pre-operative chemotherapy and radiation will be measured by comparing tumor size at time of registration with measurements taken 0-2 weeks prior to surgical resection. Response will be measured through evaluation of the MRI change, CT change, or change in physical examination. In addition, pathologic response will be recorded at the time of pathologic review of the resected specimen (*see pathology Section 10.0*).

11.2.2.1 Evaluation of target lesions-RECIST criteria

- **Complete Response (CR):** Disappearance of all target lesions as measured by MRI, CT, or physical examination. This is the order of preference for measurement.
- **Partial Response (PR):** At least a 30% decrease in the sum of the longest diameter (*LD*) of target lesions. The order of preference for measurement is MRI, CT, or physical examination.
- **Progressive Disease (PD):** At least a 20% increase in the sum of the LD of target lesions, taking as reference the smallest sum LD recorded since the treatment started or the appearance of one or more new lesions. The order of preference for measurement is MRI, CT, or physical examination.
- **Stable Disease (SD):** Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum LD since the treatment started.

11.2.2.2 Evaluation of target lesions-95-14 criteria

- **Complete response (CR):** Disappearance of all measurable tumor as measured by MRI, CT or physical examination (*PE*). This is the order of preference of items for measurement of size.
- **Partial response (PR):** 50% or greater decrease in product of perpendicular dimensions as measured on MRI, CT or PE. This is the order of preference of items for measurement of size.
- **Progression (P):** Progression is defined as an increase in size of the lesion by $> 25\%$ as defined by the product of the perpendicular dimensions on MRI, CT or PE. This is the order of preference of items for measurement of size.

11.2.3 Record of Timing of Therapies

Modification of Sugem 5416 dose associated with toxicity is noted in Section 7.3. Delays in institution of radiotherapy (*RT*) or surgery should be recorded. Ideally there will be 2 days between the completion of Sugem 5416 and surgical resection. Surgery should be completed by day 70 of protocol (*time from first radiation treatment to time of surgery*). Delay from day 70 to 80 will be considered a minor variation.

Delay beyond day 80 will be considered a major deviation. Sugren 5416 should be started at the beginning of radiation. Delay of more than 1 week will be considered a major deviation.

11.3 Wound Complications

Wound complications occurring within 120 days after surgery are to be reported. They will be categorized as noted below. These categories will also apply to the control or preoperative radiation therapy arm of the protocol. *Note: Any major wound complication in Category 2 or 3 should be reported immediately to RTOG headquarters.*

11.3.1 *Category 1:* This is a minor wound complication such as a minor skin separation or delayed drain removal. This category of complication does not result in delayed surgical resection (*see Section 11.2.2*).

11.3.2 *Category 2:* This represents a more serious problem, which seriously delays surgical resection. Included would be a major infection, but a complication in which limb loss is not threatened. This includes secondary operations required for wound treatment, deep wound packing, or invasive procedures required for wound care (*i.e. drainage of hematoma or abscess*).

11.3.3 *Category 3:* This represents a very serious infection or vascular complication in which limb loss, major soft tissue loss or skin loss is threatened. This should be accompanied by hospital admission for normal care.

11.5 Definition of Recurrence

Recurrence represents the time when recurrent or persistent disease is noted. This must be biopsy proven.

11.6 Time to Recurrence

Time to recurrence represents the time from registration to time that recurrence is biopsied.

11.7 Survival

Survival represents the time from registration to the time of death.

11.8 Follow-up

Patients will be followed until death. Follow-up must include MRI or CT scans as indicated. Every effort should be made to obtain an autopsy to document the extent of disease at the time of death.

11.9 Guidelines for MR Imaging Protocol for Musculoskeletal Sarcomas (Optional Dynamic MRI)

11.9.1 This MR imaging protocol will be performed as a pretreatment evaluation and as a measurement of response. Patients will undergo initial conventional and dynamic MR imaging within 4 weeks of start of treatment. To assess patient's response to angiogenesis inhibitor SU5416, and radiotherapy, conventional and dynamic MR imaging will be performed pre and post treatment (preoperatively). See 11.1.

11.9.2 MR imaging will be performed with a superconductive 1.5 T system using dedicated extremity, body or surface coils. Before the patient is advanced into the magnet, a 20-22 gauge cannula will be placed via antecubital vein access. The cannula will be connected to extension tubing, previously flushed with normal saline. A saline drip will keep the intravenous line open during imaging before administration of contrast material.

11.9.3 Pre-contrast imaging will include spin-echo sequences designed to demonstrate both the compartmental and longitudinal extent of the lesion. For evaluation of the effect of therapy, the following parameters will be assessed on initial and follow up MR examinations: dimensions, viability, peritumoral edema, margins, and involvement of adjacent structures (bone, joints, and neural vascular bundle). Long axis and transverse T1-weighted spin-echo sequences (TR/TE, 500-600/10-15 with two excitations and a 256 x 256 matrix) will be followed by transverse fast spin-echo T2 weighted images (TR/TR, 3500-4000/80-90 with one excitation and a 256 x 256 matrix). The choice of coil and field of view (20-40) will vary depending on the location of the lesion. The section thickness will range between 4-10 mm. with a 0.5 to 2mm intersection gap. All imaging parameters will be recorded on a report form (Appendix XII). The local investigator will retain a copy of the form. Follow-up examinations will be performed using the identical protocol. If the lesion has increased in size at the time of follow-up, additional images may be performed after replication of the pre-treatment protocol.

11.9.4 Dynamic contrast-enhanced MR imaging with parametric "First-Pass" images will be used in order to depict tissue vascularization and perfusion. To minimize sampling error and to evaluate as much of the lesion as possible, the axial imaging plane that includes the longest dimension of the lesion will be selected. The location of the axial image will be measured with reference to a skeletal landmark. The distance will be noted on the case report form (for example- 17 cm caudal to the hip joint) and all follow-up scans will be performed at the identical location.

A standard dose of gadopentetate dimeglumine (0.1 mmol per kilogram of body weight) will be injected in a bolus fashion through the patient's intravenous line (5 ml/sec). The contrast injection is immediately followed by a 20 ml saline flush at the same injection rate. For dynamic MR imaging, short T1-weighted gradient echo sequences will be used, with an acquisition time of approximately 1.41 seconds per image.

Receiver and transmitter attenuations will be kept constant for all images obtained with this sequence. Contrast material injection will be started after the fourth short T1-weighted gradient echo image. With this imaging technique, fifty sequential images will be obtained for a total acquisition time of 120 seconds (2 minutes) at one level through the lesion.

After the dynamic postcontrast sequence has been completed, long axis and transverse T1-weighted SE images will be repeated with same sequence parameters.

11.9.5 Image analysis:

1) Viability: A region of interest will be traced on the images to include the entire tumor. Whenever the status of a region is doubtful, the bias will be toward inclusion. Post-contrast images may be used to identify the area to be included. Tumor area will be recorded. Initial rates of enhancement (slope values of the contrast enhancement-time curves for the first 45 seconds) will be calculated on a pixel-by-pixel basis and displayed in a computer-generated "first pass" image, in which signal intensity reflects the slope value of the pixel.

2) Volume: Tumor size will be assessed on T2 weighted images. Tumor volume will be measured in cubic cm by tracing the tumor on each image and summation with appropriate correction for slice thickness. If the number of images demonstrating tumor is > 15, alternate images may be used with interpolation algorithms. Areas thought to represent cystic necrosis or hemorrhage will be included in the volumes.

3) Edema: Peritumoral edema will be assessed on T2-weighted images as absent, moderate, marked, or indeterminate. On follow-up imaging, T2 sequences will be assessed as unchanged, increased, decreased or indeterminate.

4) Margins: Margins between tumor and surrounding soft tissues will be assessed on initial MR imaging as infiltrating if a boundary cannot be identified over at least 25% of the interface. Non-infiltrating margins lesions will be described if the boundaries are discrete or demarcated by a pseudo-capsule. On follow-up studies margins will be classified as unchanged, more discrete, or less discrete.

5) Local extension: Involvement of adjacent bone, joints and/or major neurovascular structures will be noted on both initial imaging and follow-up imaging as: none, possible, definite or indeterminate, and the type of involvement further classified as invasion or displacement.

11.10 Guidelines for Tagged Red-blood Cell SPECT Scanning for Musculoskeletal Sarcomas^{34,35} (Optional, institution specific)

11.10.1 Patients will be assessed non-invasively with a standard gamma camera using quantitative dynamic Tc99m radiolabeled red-cell tumor perfusion scintigraphy. Since there is a direct relationship between the amount of red blood cells and the number of vessels present around and within the tumor, variation of perfusion before and after treatment should reflect the microvascular environment of the neo-endothelial vasculature around the tumor. This technique should provide for a quantitative differential analysis of microvasculature of the tumor in vivo both pre- and post-treatment. In addition, the majority of these soft tissue sarcomas are in extremity locations and background noise for visceral uptake should not be a problem. The opposite limb can serve as a standard control.

11.10.2 Background

In order to evaluate quantitatively, non-invasively, and with a procedure routinely performed in most institutions, red-blood cell labeled perfusion scintigraphy will be used to evaluate the effect of radiation therapy and SU5416 on extremity soft tissue sarcomas with a three phase red-cell scintigraphy study before and after therapeutic intervention. This approach is based on the assumption that radiation therapy combined with the VEGF tyrosine kinase inhibitor will adversely effect tumor and peritumoral neovascularity and hence red blood cell accumulation.

11.10.3 Procedure

A red cell scintigraphy with an angiographic blood pool and delayed phase on the extremity sarcoma should be performed before either RT alone or RT and SU5416 and then repeated upon completion of the preoperative therapy 1 week before surgery. The patient's own red-blood cells will be labeled in vivo with 15-20 mCi of Tc-99m using the ultratag RBC kit as routinely performed for blood pool imaging in nuclear medicine. The labeled red cells will then be injected intravenously and dynamic angiographic images of the tumor will be taken at a rate of 1 frame per second over a 2-minute period using a 128*128 word mode matrix in the anterior or posterior projection depending on the position of the tumor within the extremity. A 2-minute image of the injection site will then be taken using a 128*128 word mode matrix to verify the presence or absence of subcutaneous infiltration. A blood pool image of 10 minutes will then be acquired 10 minutes after the injection in the anterior and posterior projection using a 128*128 word mode matrix. A

10 minute delayed image will be acquired 30 minutes and 60 minutes after the injection in both projections using the same matrix.

11.10.4 Processing End-Points

The aim of the study is to compare intraindividually the variation of red cell accumulation within the tumor before and after radiation therapy alone or combined with SU5416. Perfusion data will be used to assess the adequacy of the bolus; the blood pool images will help to determine red cell accumulation variation over the first hour. The 30 and 60-minute images will be used to determine the tumor activity. The time activity curves of the main supplying artery and tumor will be generated using the angiographic images; the slopes of the ascending portion of the main supplying artery and tumor time activity curves will be visually compared. The tumor slope will be labeled as being either parallel, decreased, or delayed in comparison to the supplying slope. Blood pool and delayed images will be used to determine the geometric mean (*the square root of the anterior*posterior counts*), background and injected dose corrected activity per pixel within the tumor before and after therapy. We will call this parameter the SSUV for sarcoma standardized uptake value. An intraindividual variation of more than 10% will be considered as significant.

11.10.5 Any questions concerning use of radiolabeled red cell scintigraphy for this perfusion study or the interpretation of the data should be directed towards Dr. Jean Luc Urbain: FAX # (215) 379-5717; office phone (215) 728-3041, e-mail: JL_Urbain@fccc.edu.

11.10.6 Data Collection Sheet (*See Appendix VI*).

12.0 DATA COLLECTION

(RTOG, 1101 Market Street, Philadelphia, PA 19107, FAX#215/928-0153)

12.1 Summary of Data Submission

<u>Item</u>	<u>Due</u>
Demographic Form (A5)	Within 2 weeks of study entry
Initial Evaluation Form (I1)	
Pathology Report (P1)	
Pathology Slides/Blocks (P2)	
Specimen Transmittal (ST)	
 <u>Final Dosimetry Information:</u>	 At completion of all RT
Pre-op CT/MRI Scan with tumor volume outlined (C1)	
Pre-op CT/MRI Scan Report (C3)	
Radiotherapy Form (T1)	
Daily Treatment Record (T5)	
Isodose Distribution (T6)	
Boost Films (<i>simulation and portal</i>) (T8)* (if applicable)	
Calculation Form (TL)	
Simulation and Port (TP)	
 Specimen Transmittal (ST)	 At midpoint of Sugem 5416 treatments
 Sugem 5416 Flowsheets (TF)	 At completion of Sugem 5416 treatments
 Post-RT +/- SU5416 Evaluation Form (F0) before surgery	 <u>Mail within 48 hours of re-evaluation</u>
 Specimen Transmittal (ST)	 On day of surgery, pre-op specimen
 Surgery Form (S1)	 Within two weeks of surgery
Operative Notes (S2)	
Surgical Pathology Reports (S5)	
Pathology Slides/Blocks (P2)	
Perioperative Questionnaire (PO)	Four weeks after surgery
 Specimen Transmittal (ST)	 Six weeks post-op specimen
 Follow-up Form (F1) years; q 6 months years 2-5 years, then annually. Also at progression/relapse and at	 Every 3 months from end of treatment for 2 death.
 Long Term Follow-up Form (FF)	 Yearly after 5 years in place of the F1 form, as applicable. See FF Form for instructions.
 Autopsy Report (D3)	 As applicable

12.2 Dosimetry Submission

12.2.1 Dosimetry data must include complete and final information for patients receiving post operative RT. These include completed T5, T6 as well as T8 (*boost films*). **Patients who do not receive post operative RT do not require T8 films**. All dosimetry data will be submitted directly to RTOG.

13.0 STATISTICAL CONSIDERATIONS

13.1 Study Endpoints

- 13.1.1** Dose limiting toxicities (*DLT*) using the common toxicity criteria (*CTC*) Version 2.0 will be defined as one of the following:
- a) Grade 3 or 4 nonhematologic (*including hepatic*);
 - b) Grade 4 neutropenia or thrombocytopenia due to SU5416;
 - c) Fatal toxicity.
- 13.1.2** Other toxicities
- 13.1.3** Wound complications
- 13.1.4** Tumor markers
- 13.1.5** Disease-free survival
- 13.1.6** Local recurrence, distant metastases, and overall survival rates.

13.2 Overview

The goal of this study is twofold. It is to first establish that SU5416 at the dose of 145 mg/m² added to pre-operative radiation therapy can be delivered safely. The criteria will be that no patients will develop fatal (*grade 5*) toxicity and less than 50% of patients will develop dose limiting toxicities. The determination of the dose limiting toxicities (*Section 13.1.1*) will occur prior to surgery. If, at any time, Grade 5 toxicity is observed, then accrual will be suspended for that dose level, and the Study Chair will review the event. If death is judged to be treatment-related by the study chair, the next lower dose will be judged to be the MTD. Past RTOG experience with phase I studies has shown that re-starting patient accrual after its suspension to evaluate dose toxicity can take some time. In this study, there will be no suspension of patient accrual. Instead, patients will be registered to the RT alone arm in the interim between SU5416 dose levels. Based upon the closed sarcoma study RTOG 95-14, the monthly accrual rate is anticipated to be 1.5 cases per a month. If a dose level, on which patients are still being treated, is determined to be too toxic, their treating clinician will be immediately contacted to lower the dose. Once the dose question is answered, additional patients will be entered to generate pilot data on whether SU5416 has quantitated anti-angiogenic effects and has synergistic response when combined with radiation therapy.

13.3 Phase I Component

13.3.1 Evaluation of Toxicity

Patients will be carefully evaluated with respect to dose limiting toxicities prior to surgery.

13.3.2 Dose Escalation Scheme and Sample Size

Six patients will be entered at the current dose level. While toxicity is being evaluated at this dose level, patients will be entered onto the RT only arm. Toxicity will be evaluated in cohorts of 3 patients. First, toxicity will be evaluated in the first 3 patients. If 0/3 patients have DLT, then the current dose level will be considered acceptable. If 1/3 patients have DLT, then the next 3 will be evaluated. If 0/3 of this next cohort have DLT, then the dose will be considered acceptable. If, at any time, 2 patients at the current dose level have DLT, then the dose level will be considered unacceptable. If the dose level is considered acceptable, the next 6 patients will be entered to the next higher dose and toxicity will be evaluated in the same manner. Again, patients will be entered onto the RT only arm in the interim. If a dose level is considered unacceptable, the next lower dose that has been determined acceptable will be considered the MTD. If the first dose level (110 mg/m²) is considered unacceptable, then 6 patients will be entered at 85 mg/m² and this dose will be evaluated. At a given dose level, the probability of halting dose escalation when the true toxicity is 50% of higher is greater than 82%. In addition, if the true toxicity is instead 20%, then there will be only a 29% probability of discontinuing dose escalation at a given dose level.

Once the MTD is established the phase II component will begin.

13.3.3 Patient Accrual

The patient accrual is projected to be 1.5 patients per month. The phase I part is projected to take approximately 12 months to complete. **It is expected that total accrual during the phase I component will be approximately 18 patients (6 to each dose level, and 6 to RT only).**

13.3.4 Analysis Plan

13.3.4.1 Interim Reports

Interim reports will be prepared every six months in the RTOG pre-meeting book until decision about safety of 145 mg/m² is established. In general, the interim reports include information about accrual rate, pretreatment characteristics of patients accrued, and the frequencies and severity of toxicity.

13.3.4.2 Reporting the Phase I Results

The analysis will be undertaken shortly after decision about safety of 145 mg/m² is established. The usual components of this analysis are: patients excluded from the analyses with their reasons for exclusion; institutional accrual; distribution of the important baseline prognostic variables, and observed results with respect to the endpoints described in Section 13.1.1 – 13.1.3.

13.4 Phase II Component

13.4.1 Sample Size and Patient Accrual

The second component of this study is to generate data concerning anti-angiogenic effects of SU 5416 as stated in objectives 2.2 and 2.3. The intention here would be to generate hypotheses for more definitive testing. It is anticipated that accrual should increase to about 2 patients per month in the phase II component. In order to evaluate the angiogenesis effects of SU5416, we will need to evaluate 20 patients treated with RT + SU5416 (at the MTD) and 20 patients treated with RT only. The patients treated in the phase I component will be counted against these totals. Patients will be randomized to RT+ SU5416 or RT only, but the randomization will be unequal (*if necessary*), and will continue until each arm has accrued 20 patients. The proportion of patients randomized to each arm will be determined by the number of patients necessary to complete the phase I component of the protocol. For example, if both the first dose level and the second dose level require 6 patients each to determine the MTD, and 6 patients are assigned to RT only in the interim, then 14 additional patients will be randomized to RT only, and 14 patients will be randomized to RT + SU5416 at the MTD. If, however, only 4 patients are assigned to the RT only arm in the phase I component, then 14 patients will be randomized to the RT and SU5416 arm, and 16 to the RT only arm.

Total accrual during the phase II component will be approximately 28 patients.

13.4.2 Analysis Plan

13.4.2.1 Interim Reports

Interim reports will be prepared every six months until the final analysis. In general, the interim reports include information about accrual rate with projected completion date, pretreatment characteristics of patients accrued, quality of submitted data with respect to timeliness, completeness, and accuracy, compliance rate of treatment delivery with respect to the protocol prescription, the frequencies and severity of toxicity.

13.4.2.2 Reporting the Phase II Results

This major analysis will be undertaken when each patient has been potentially followed for a minimum of 24 months. The usual components of this analysis are: patients excluded from the analyses with their reasons for exclusion; institutional accrual; distribution of the important baseline prognostic variables, patient accrual rate with projected completion date, observed results with respect to the endpoints described in Section 13.1.

13.5 NIH Requirement

13.5.1 In conformance with the National Institute of Health (*NIH*) Revitalization Act of 1993 with regard to inclusion of women and minority in clinical research, we have considered the two possible interactions (*treatment by race and treatment by gender*). The study was designed under the assumption of the same tolerance and efficacy across the genders and across the races. A statistical analysis will not be performed to examine the possible difference between the genders and among the races because of the rather small sample size. The following table gives the projected number of patients in each race and gender group based upon RTOG 95-14.

Projected Gender and Minority Inclusion

	American Indian or Alaskan Native	Asian	Black or African American	Hispanic or Latino	Native Hawaiian or Pacific Islander	White	Other or Unknown	Total
Female	0	0	2	0	0	16	0	18
Male	0	0	8	0	0	20	0	28
Total	0	0	10	0	0	36	0	46

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APPENDIX I

RTOG S-0120

SAMPLE CONSENT FOR RESEARCH STUDY

A PHASE I/II STUDY OF PREOPERATIVE RADIOTHERAPY WITH/WITHOUT SUGEN 5416 (A *TK INHIBITOR ANTI-ANGIOGENESIS COMPOUND*) IN THE MANAGEMENT OF LOW TO INTERMEDIATE GRADE SOFT TISSUE SARCOMA OF THE TRUNK OR EXTREMITY

This is a clinical trial (a type of research study). Clinical trials include only patients who choose to take part. Please take your time to make your decision. Discuss it with your friends and family. The National Cancer Institute (NCI) booklet, "Taking Part in Clinical Trials: What Cancer Patients Need To Know," is available from your doctor.

You are being asked to take part in this study because you have soft tissue sarcoma of the trunk or extremity.

WHY IS THIS STUDY BEING DONE?

Delivering radiation before surgery improves tumor control in patients with soft tissue sarcoma. This is generally considered standard therapy. The primary purpose of this study is to see whether the addition of the investigational agent Sugem 5416 with the radiation therapy will improve the tumor response seen by your doctors (becoming smaller during treatment).

This research is being done to see the effects that this treatment will have on controlling the cancer. In addition, your physicians hope to learn whether this therapy changes wound healing. The tumor tissue that is removed will also be studied to learn more about how these tumors grow and function and specifically how this new medicine may affect the small blood vessels supplying the tumor.

This study consists of two parts. In the Phase I portion of the study, patients will receive the investigational agent, Sugem 5416, in addition to radiation, or patients will receive radiation alone. The highest dose of Sugem 5416 that can be administered with radiation therapy will be determined. Patients will receive between 110-145 mg/m² of Sugem 5416. This portion of the study requires that Sugem 5416 be increased only after safety at the previous dose has been confirmed in multiple patients. In the Phase II portion of this study, patients will receive either radiation therapy alone or radiation therapy plus the safest tolerated dose of Sugem 5416 (determined in Phase I).

HOW MANY PEOPLE WILL TAKE PART IN THE STUDY?

Between 40-46 people will take part in this study.

WHAT IS INVOLVED IN THE STUDY?

You will receive either radiation therapy (RT) alone prior to surgery or RT and drug (given twice weekly for approximately 8 weeks) prior to surgery. About two weeks later, you will have the tumor removed surgically. There will be approximately 2-1/2 months between the start of the radiation and the surgery. The surgery will be planned to remove the complete tumor. The drug used in this study is called Sugen 5416, which will be supplied to you at no cost.

If you receive the drug, the dosage of the drug that you receive will depend on how many patients have entered the study before you. The first few patients entered on the study will receive a dose of drug that previous research suggests will be safe. If they have no serious problems, the next patients receive a higher dose.

Each cycle of drug is given over one hour through a tube in your vein (i.v.) twice a week. Patients will be observed for a minimum of three hours during the first three administrations of SU5416. Both radiation and drug will be given as an outpatient. The radiation treatments take a few minutes once a day, Monday through Friday, over five weeks. The following table shows the planned schedule:

Treatment	Radiation Days	SU5416 and Radiation Days	SU5416 Days After Completion of Radiation	Surgery Day	Postoperative Radiation^c
Sugen 5416		2,4,9,11,16,18, 23,25,30,32	37,39,44, 46, 51,53,58,60 ^a		84 ^d
RT	1-5, 8-12, 15-19, 22-26, 29-33				84 ^d
Surgery				48-62 ^b	

- Dose number dependent on date of surgery. SU5416 to stop two days prior to surgery.
- Surgery to be done within this time range.
- Two weeks of radiation for positive surgery margins only.
- To begin two weeks after surgery if healing is adequate.

Further radiation may be required after your surgery if some tumor cells are present at the edges of the removed tissue. If you received drug before surgery, you will also receive it if further radiation is required after your surgery. It will be given at the same dose as before your surgery.

This most likely would take place two weeks after your surgery and take two weeks to complete.

The tumor tissue's cells will be examined to see if any special "markers" (tests which predict how a patient with tumors like yours responds to treatment) can be identified.

If you take part in this study, you will have the following tests and procedures:

Prior to study entry:

- Incisional or core biopsies
- History and physical

- Chest CT & X-ray
- MRI or CT of tumor
- Blood tests
- Pregnancy test (if applicable)
- EKG

During radiation/drug therapy:

- History and physical
- Blood tests

Before surgery:

- History and physical
- Chest CT
- MRI or CT of tumor
- Blood tests

At completion of treatment:

- History and physical
- Chest CT
- MRI or CT of tumor
- Blood tests

During follow-up:

- History and physical – every 3 months for the first 2 years, every 6 months for years 2-5, and then yearly after that
- Chest CT, MRI/CT of tumor – every 6 months following treatment
- Chest X-ray – every 3 months for first 2 years; yearly after 5 years

Also, at the time of your diagnosis by biopsy, some of your tumor was removed. As is usually done, this tissue went to the hospital's pathology department for routine testing and diagnosis. After that process was complete, the remaining tumor samples were stored in the pathology department. You are being asked for permission to use the remainder of the tumor samples for additional tests. Since this tissue was removed at the time of surgery or biopsy, your permission to use this tissue will not lead to any additional procedures or expense. This tissue will be sent to a central office for review and research investigation associated with this protocol. In addition, several blood samples will be taken during your treatment and sent for testing to evaluate the effect of the treatment on the tumor blood vessels.

HOW LONG WILL I BE IN THE STUDY?

This study will take approximately three months to complete. Follow-up visits will be scheduled every 3 months for two years, every six months for years 2-5, and then yearly after 5 years.

The researcher may decide to take you off this study if it is in your medical best interest, your condition worsens, or new information becomes available and this information suggests the treatment will be

ineffective or unsafe for you. It is unlikely, but the study may be stopped due to lack of funding or participation.

You can stop participating at any time. However, if you decide to stop participating in the study, we encourage you to talk to the researcher and your regular doctor first.

WHAT ARE THE RISKS OF THE STUDY?

While on the study, you are at risk for these side effects. You should discuss these with the researcher and/or your regular doctor. There also may be other side effects that we cannot predict. Other drugs will be given to make side effects less serious and uncomfortable. Many side effects go away shortly after the drug and radiation are stopped, but in some cases side effects can be serious or long-lasting or permanent.

Risks Associated with Sugen 5416

Very Likely

Headaches
Nausea and vomiting
Diarrhea – change in bowel habits
Abdominal pain
Weight loss
Loss of muscle mass
Fatigue

Less Likely

Sore throat
Skin rash and swelling
Males (decreased libido and sperm count)

Less Likely But Serious

Blood clots in legs, lungs, brain, or liver
Shortness of breath, cough
Pneumonia
Low blood pressure
Allergic reaction
Heart damage
Bleeding
Elevation of liver blood tests and liver dysfunction; recent report of liver rupture in patients with metastatic cancer
Increased calcium in blood
Damage to kidneys

Radiation Therapy

The risks and discomforts associated with radiation can be divided into early reactions (*those happening during or shortly after radiation*) and late reactions (*those happening well after the completion of radiation*). In general, most radiation reactions (*other than fatigue*) are limited to the site being treated. For example, if your leg is being treated, you will not feel nauseated from radiation treatment. Your doctor will specifically identify those risks connected with the location of your tumor.

Very Likely:

Reduction in blood counts-radiation can temporarily lower red blood cell, white cell and platelet counts possibly resulting in bleeding and infection

Reddening or tanning of the skin

Hair loss in the treatment area

Nausea/Vomiting

Loss of appetite

Weight loss

Fatigue and weakness

Diarrhea

Delayed wound healing after surgery

Less Likely:

Pain and swelling

Fracture – radiation can make bones more susceptible to fracture

Bruising – skin subject or radiation may heal more slowly if injured or bruised

Less Likely But Serious:

Bowel injury if the abdominal wall is being treated

Spinal cord injury if the back area is being treated

Risk of cancer – radiation can cause tumors in treated tissues

Damage to other organs – if heart, lung, liver or stomach are in the field of treatment these organs can be damaged

Reproductive risks: Because the drug in this study can affect an unborn baby, you should not become pregnant or father a baby while on this study. You should not nurse your baby while on this study. Ask about counseling and more information about preventing pregnancy.

ARE THERE BENEFITS TO TAKING PART IN THE STUDY?

If you agree to take part in this study, there may or may not be direct medical benefit to you. We hope the information learned from this study will benefit other patients with soft tissue sarcoma in the future.

WHAT OTHER OPTIONS ARE THERE?

You may choose to not participate in this study. Other treatments that could be considered for your condition may include the following: (1) radiation therapy; (2) chemotherapy; (3) surgery; or (4) no treatment except medications to make you feel better. With the latter choice, your tumor would continue to grow and your disease would spread. These treatments could be given either alone or in combination with each other.

Your doctor can tell you more about your condition and the possible benefits of the different available treatments.

WHAT ABOUT CONFIDENTIALITY?

Efforts will be made to keep your personal information confidential. We cannot guarantee absolute confidentiality. Records of your progress while on the study will be kept in a confidential form at this institution and in a computer file at the headquarters of the Radiation Therapy Oncology Group (*RTOG*). Your personal information may be disclosed if required by law.

Organizations that may inspect and/or copy your research records for quality assurance and data analysis include groups such as the Food and Drug Administration (*FDA*), the National Cancer Institute (*NCI*), qualified representatives of applicable drug manufacturers, and other groups or organizations that have a role in this study.

WHAT ARE THE COSTS?

The Division of Cancer Treatment and Diagnosis, NCI, will provide you with the investigational agent free of charge for this study. Sugem 5416 will be provided at no cost to you. Every effort has been made to insure adequate supplies of the investigational agent for this study. If, however, the investigational agent becomes commercially available while you are being treated, there is a possibility that you would be asked to purchase subsequent supplies. Taking part in this study may lead to added costs to you or your insurance company. Please ask about any expected added costs or insurance problems.

In the case of injury or illness resulting from this study, emergency medical treatment is available but will be provided at the usual charge. No funds have been set aside to compensate you in the event of injury.

You or your insurance company will be charged for continuing medical care and/or hospitalization.

You will receive no payment for taking part in this study.

WHAT ARE MY RIGHTS AS A PARTICIPANT?

Taking part in this study is voluntary. You may choose not to take part or may leave the study at any time. Leaving the study will not result in any penalty or loss of benefits to which you are entitled.

We will tell you about new information that may affect your health, welfare, or willingness to stay in this study.

WHOM DO I CALL IF I HAVE QUESTIONS OR PROBLEMS?

(This section must be completed)

For information about your disease and research-related injury, you may contact:

Name

Telephone Number

For information about this study, you may contact:

Name

Telephone Number

For information about your rights as a research subject, you may contact:

(OPRR suggests that this person not be the investigator or anyone else directly involved with the research)

Name

Telephone Number

WHERE CAN I GET MORE INFORMATION?

You may call the NCI's Cancer Information Service at
1-800-4-CANCER (1-800-422-6237) or TTY: 1-800-332-8615

Visit the NCI's Web sites for comprehensive clinical trials information
<http://cancertrials.nci.nih.gov> or for accurate cancer information including PDQ
<http://cancernet.nci.nih.gov>.

SIGNATURE

I have read all the above, asked questions, and received answers concerning areas I did not understand. I have had the opportunity to take this consent form home for review or discussion.

I willingly give my consent to participate in this program. Upon signing this form I will receive a copy. I may also request a copy of the protocol (*full study plan*).

Patient Signature (*or legal Representative*)

Date

TISSUE AND BLOOD TESTING (RTOG S-0120)

I agree to the use of my tissues/other samples for research studies related to my cancer.

Yes No

Patient Signature (*or legal Representative*)

Date

APPENDIX II

KARNOFSKY PERFORMANCE SCALE

- 100 Normal; no complaints; no evidence of disease
- 90 Able to carry on normal activity; minor signs or symptoms of disease
- 80 Normal activity with effort; some sign or symptoms of disease
- 70 Cares for self; unable to carry on normal activity or do active work
- 60 Requires occasional assistance, but is able to care for most personal needs
- 50 Requires considerable assistance and frequent medical care
- 40 Disabled; requires special care and assistance
- 30 Severely disabled; hospitalization is indicated, although death not imminent
- 20 Very sick; hospitalization necessary; active support treatment is necessary
- 10 Moribund; fatal processes progressing rapidly
- 0 Dead

ZUBROD PERFORMANCE SCALE

- 0 Fully active, able to carry on all predisease activities without restriction (*Karnofsky 90-100*).
- 1 Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature. For example, light housework, office work (*Karnofsky 70-80*).
- 2 Ambulatory and capable of all self-care but unable to carry out any work activities. Up and about more than 50% of waking hours (*Karnofsky 50-60*).
- 3 Capable of only limited self-care, confined to bed or chair 50% or more of waking hours (*Karnofsky 30-40*).
- 4 Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair (*Karnofsky 10-20*).

New York Heart Association Functional Status	
Class I	No symptoms
Class II	Symptoms with moderate or severe activity
Class III	Symptoms with mild sedentary living
Class IV	Symptoms at rest

APPENDIX III - A

STAGING SYSTEM (AJCC, 5th edition - 1998)

DEFINITION OF TNM

Primary Tumor (T)

- TX Primary tumor cannot be assessed
- T0 No evidence of primary tumor
- T1 Tumor 5 cm or less in greatest dimension
 - T1a Superficial
 - T1b Deep
- T2 Tumor more than 5 cm in greatest dimension
 - T2a Superficial
 - T2b Deep

Regional Lymph Nodes (N)

- NX Regional lymph nodes cannot be assessed
- N0 No regional lymph node metastasis
- N1 Regional lymph metastasis

Regional Lymph Nodes. The regional lymph nodes are those appropriate to the site of the primary tumor.

Unilateral Tumors

Head and neck	Ipsilateral preauricular, submandibular, cervical, and supraclavicular lymph nodes
Thorax	Ipsilateral axillary lymph nodes
Arm	Ipsilateral epitrochlear and axillary lymph nodes
Abdomen, loins and buttocks	Ipsilateral inguinal lymph nodes
Leg	Ipsilateral popliteal and inguinal lymph nodes
Anal margin and perianal skin	Ipsilateral inguinal lymph nodes

Distant Metastasis (M)

- MX Presence of distant metastasis cannot be assessed
- M0 No distant metastasis
- M1 Distant metastasis

Metastatic Sites. The lung is the most common site, but any body site may be involved.

STAGE GROUPING

Stage IA	G1-2	T1a-1b	N0	M0
Stage 1B	G1-2	T2a	N0	M0
Stage IIA	G2	T2b	N0	M0
Stage IIB	G3-4	T1a-1b	N0	M0
Stage IIC	G3-4	T2a	N0	M0
Stage III	G3-4	T2b	N0	M0
Stage IV	Any G	Any T	N1	M0
	Any G	Any T	Any N	M1

HISTOPATHOLOGIC GRADE (G)

After the histologic type has been determined, the tumor should be graded according to the accepted criteria of malignancy, including cellularity, cellular pleomorphism, mitotic activity, and necrosis. The amount of intercellular substance, such as collagen or mucoid material, should be considered as favorable in assessing grade.

- GX Grade cannot be assessed
- G1 Well differentiated
- G2 Moderately differentiated
- G3 Poorly differentiated
- G4 Undifferentiated

APPENDIX III - B

Enneking System for Staging of Sarcomas of Soft Tissues or Bone

<u>Stage</u>	<u>Characteristic</u>
I	<u>Low Grade</u>
IA	Intracompartmental
IB	Extracompartmental
II	<u>High Grade</u>
IIA	Intracompartmental
IIB	Extracompartmental
III	<u>Any Grade</u>
	N1 or M1

APPENDIX V

ADVERSE EVENT REPORTING GUIDELINES

A. GENERAL GUIDELINES

In order to assure prompt and complete reporting of toxicities, the following general guidelines are to be observed. These apply to all RTOG studies and Intergroup Studies in which RTOG participates. **When a protocol toxicity requires more intense, special handling, study-specific reporting procedures supersede the General Guidelines.**

1. The Principal Investigator will report the details of any unusual, significant, fatal or life-threatening protocol treatment reaction to the RTOG Group Chairman and to the Headquarters Data Management Staff (215/574-3214) within 24 hours of discovery. When telephone reporting is required, the Principal Investigator should have all relevant material available. See the protocol-specific criteria to grade the severity of the reaction.
 - a. All deaths during protocol treatment or within 30 days of completion or termination of protocol treatment regardless of cause requires telephone notification within 24 hours of discovery.
2. The Principal Investigator will also report the details of the significant reaction to the Study Chairman by telephone.
3. A written report, including all relevant study forms, containing all relevant clinical information concerning the reported event will be sent to RTOG Headquarters by the Principal Investigator. This must be sent within 10 working days of the discovery of the toxicity unless specified sooner by the protocol (FAX #215/928-0153).
4. The Group Chairman in consultation with the Study Chairman will take appropriate and prompt action to inform the membership and statistical personnel of any protocol modifications and/or precautionary measures if this is warranted.
5. For those incidents requiring telephone reporting to the National Cancer Institute (NCI), Investigational Drug Branch (IDB) or Food and Drug Administration (FDA), the Principal Investigator should first call RTOG (as outlined above) unless this will unduly delay the notification process required by the federal agencies.

A copy of all correspondence submitted to NCI, or to another Cooperative Group (in the case of RTOG-coordinated intergroup studies) must also be submitted to RTOG Headquarters when applicable.

6. The Principal Investigator, when participating in RTOG-coordinated Intergroup studies, is obligated to comply with all additional reporting specifications required by an individual study.
7. Institutions must also comply with their individual Institutional Review Board policy with regard to toxicity reporting procedure.
8. Failure to comply with reporting requirements in a timely manner may result in suspension of patient registration.

B. RADIATION TOXICITY GUIDELINES

1. All fatal toxicities (grade 5) resulting from protocol treatment must be reported by telephone to the Group Chairman, to RTOG Headquarters Data Management and to the primary Study Chairman within 24 hours of discovery.

2. All life-threatening (*grade 4*) toxicities resulting from protocol treatment using non-standard fractionated treatment, brachytherapy, radiopharmaceuticals and radiosurgery must be reported by telephone to the Group Chairman, to RTOG Headquarters Data Management and to the primary Study Chairman within 24 hours of discovery.
3. Appropriate data forms, and if requested a written report, must be submitted to Headquarters within 10 working days of the telephone report.

C. ADVERSE DRUG REACTIONS - DRUG AND BIOLOGICS

An adverse reaction is a toxicity or an undesirable effect usually of severe nature. Specifically, this may include major organ toxicities of the liver, kidneys, cardiovascular system, central nervous system, skin, bone marrow, or anaphylaxis. These undesirable effects may be further classified as "known" or "unknown" toxicities.

Known toxicities are those which have been previously identified as having resulted from administration of the agent. They may be identified in the literature, the protocol, the consent form or noted in the drug insert.

Unknown toxicities are those thought to have resulted from the agent but have not previously been identified as a known side effect.

Commercial and Non-Investigational Agents

- i. Any fatal (*grade 5*) or life threatening (*grade 4*) adverse reaction which is due to or suspected to be the result of a protocol drug must be reported to the Group Chairman or to RTOG Headquarters' Data Management Staff and to the Study Chairman by telephone within 24 hours of discovery. Known grade 4 hematologic toxicities need not be reported by telephone.
- ii. Unknown adverse reactions (\geq *grade 2*) resulting from commercial drugs prescribed in an RTOG protocol are to be reported to the Group Chairman or RTOG Headquarters' Data Management, to the Study Chairman and to the IDB within 10 working days of discovery. FDA Form 3500 is to be used in reporting details. All relevant data forms must accompany the RTOG copy of Form 3500.
- iii. All neurotoxicities (\geq *grade 3*) from radiosensitizer or protector drugs are to be reported within 24 hours by phone to RTOG Headquarters and to the Study Chairman.
- iv. All relevant data forms must be submitted to RTOG Headquarters within 10 working days on all reactions requiring telephone reporting. A special written report may be required.

Reactions definitely thought not to be treatment related should not be reported, however, a report should be made of applicable effects if there is a reasonable suspicion that the effect is due to protocol treatment.

Investigational Agents

Prompt reporting of adverse reactions in patients treated with investigational agents is mandatory. Adverse reactions from NCI sponsored drugs are reported to:

Investigational Drug Branch (*IDB*)
P. O. Box 30012
Bethesda, MD 20824
Telephone number available 24 hours
(301) 230-2330 FAX # 301-230-0159

i. Phase I Studies Utilizing Investigational Agents

- All deaths during therapy with the agent. Report **by phone** within 24 hours to IDB and RTOG Headquarters.
**A written report to follow within 10 working days.
- All deaths within 30 days of termination of the agent. As above
- All life threatening (*grade 4*) events which may be due to agent. As above
- First occurrence of any toxicity (*regardless of grade*). Report by **phone within 24 hours** to IDB drug monitor and RTOG Headquarters.
**A written report may be required.

ii. Phase II, III Studies Utilizing Investigational Agents

- All fatal (*grade 5*) and life threatening (*grade 4*) known adverse reactions due to investigational agent. Report **by phone** to RTOG Headquarters and the Study Chairman within 24 hours
**A written report must be sent to RTOG within 10 working days with a copy to IDB.
(*Grade 4 myelosuppression not reported to IDB*)
- All fatal (*grade 5*) and life threatening (*grade 4*) unknown adverse reactions resulting from or suspected to be related to investigational agent. Report **by phone** to RTOG Headquarters, the Study Chairman and IDB within **24 hours**.
**A written report to follow within 10 working days.
- All grade 2, 3 unknown adverse reactions resulting from or suspected to be related to investigational agent. **Report **in writing** to RTOG Headquarters and IDB within 10 working days.

** See attached (*if applicable to this study*) NCI Adverse Drug Reaction Reporting Form

RTOG STUDY S-0120 Case # _____
Copy to RTOG, ATTN: ADR ADR # _____
1101 Market Street
Philadelphia, PA 19107 FAX 215-928-0153

(Assigned at NCI)

DCT ADVERSE REACTION FORM FOR INVESTIGATIONAL AGENTS

Person Completing this Form _____ Date _____

Phone (____) _____

Physician Responsible for this Report _____

(Please print or type)

I. DEMOGRAPHICS

A. Patient Information

PT I.D.# _____ Age _____ Sex _____ Date of Initial Dx _____

Malignancy _____

Site of Primary _____ PS (at start of study) _____

Site(s) of Metastatic Disease _____

Concurrent Non-Malignant Disease and Non-Protocol Medications _____

B. Drug Information

Drug Name _____

Source of Drug: NCI _____ Other (specify) _____

Type of Reaction _____ Toxicity Grade _____

Date of Reaction _____ Date IRB Notified _____

NCI Protocol # _____ Attending Physician (Investigator) _____

Phase of Study _____ Institution _____ Phone (____) _____

Protocol Treatment (include all agents)

<u>Drug</u>	<u>Dose</u>	<u>Schedule</u>	<u>Route</u>
-------------	-------------	-----------------	--------------

Date First Course Started _____ Number of Courses _____

Date Last Course Started _____ Date of Therapy Associated with ADR _____

Prior Therapy (Drug, radiation, relevant surgery: Include dates of therapy) _____

II. DOCUMENTATION OF REACTION

A. Non-Myelosuppressive Toxicity and Previously Unknown Myelosuppression

1. Description of Reaction and Temporal Relationship to Investigational Drug Administration
2. Physical Findings and Laboratory Data (e.g., bilirubin, creatinine, including baseline, worst and recovery value) Documenting Toxicity
3. Treatment of Adverse Reaction
4. Past History of Organ Dysfunction
5. Rechallenge with Agent _____ No _____ Yes
If yes: _____ with reaction; describe _____

_____ without reaction

6. Patient outcome: _____ Recovered without sequelae
 _____ Recovered with sequelae; describe _____
 _____ Remains under treatment
 _____ Died; From _____ ADR _____ Malignancy _____ Other _____
 Autopsy date _____

B. Myelosuppression (Previously known or unknown)

1. Laboratory Data Documenting Myelosuppression

	<u>Baseline</u> Date/Value	<u>Nadir</u> Date/Value	<u>Recovery or Latest Value</u> Date/Value
WBC or PMN	_____/____	_____/____	_____/____
Platelets	_____/____	_____/____	_____/____
Hgb or Hct	_____/____	_____/____	_____/____

2. Complications, Treatment and Sequelae (e.g., infections/hemorrhage)

C. Grade of Toxicity and Reporting Requirements (Check one)

1. Previously Unknown Toxicities:
 a. Fatal _____ or Life-threatening _____ (Report by telephone within 24 hours: 301-230-2330) Date _____
 NCI contact _____
 b. Grade I _____ II _____ III _____ (Send form within 10 days)
2. Previously Known Non-Myelosuppressive Toxicities:
 a. Fatal _____ or Life-threatening _____ (Send form within 10 days)
3. Previously Known Myelosuppressive Toxicities:
 a. Fatal _____ (Send form within 10 days)

Send Forms to: Investigational Drug Branch, NCI
 Post Office Box 30012
 Bethesda, Maryland 20824
 FAX # 301-230-0159

D. Investigator's Assessment (If more than 1 investigational agent was used, give an assessment for each by writing the drug names on the appropriate lines.)

	IND Drug	Non-IND Drug	Disease	Action Taken:	Therapy Required:
Unrelated	_____	_____	_____	None _____	None _____
Unlikely _____	_____	_____	_____	Dose Reduced _____	Symptomatic _____
Possible	_____	_____	_____	Dose Withheld _____	Supportive _____
Probable	_____	_____	_____	Drug Discontinued _____	Intensive _____
Definite	_____	_____	_____		

E. I hereby certify that the information on this form is correct and complete to the best of my knowledge.

_____ M.D. _____
 (SIGNATURE OF RESPONSIBLE PHYSICIAN) (DATE)

APPENDIX VI

SPECT RBC PERFUSION SCAN DATA FORM

PATIENT ID# Date of Study Dose Infiltration (circle)				
	PRE-TREATMENT		POST-TREATMENT	
	Yes	No	Yes	No
	ANTERIOR VIEW	POSTERIOR VIEW	ANTERIOR VIEW	POSTERIOR VIEW
ANGIOGRAPHIC PHASE				
Tumor Perfusion Slope vs main Supplying Artery Perfusion Slope parallel decreased delayed				
PIXEL AVERAGE ACTIVITY				
30" IMAGE Blood pool Image 60" IMAGE Delayed Image				

APPENDIX VII

Project 1 Microvessel Density as a Marker of Tumor-Associated Angiogenesis and Treatment Response in Patients with Soft Tissue Sarcomas

**James C. Watson, M.D., Principal Investigator
Fox Chase Cancer Center**

Quantification of microvessel density (MVD) in histologic sections remains the most objective and reliable means of assessing tumor-associated angiogenesis¹. Numerous studies indicate that MVD associated with primary solid tumors correlates with increased metastasis or recurrence^{ii,iii,iv}, but its utility as a prognostic indicator in this disease remains in question^{v,vi}.

SPECIFIC AIMS

To quantify MVD associated with human adult soft tissue sarcomas both before and after treatment in order to accomplish the following:

- 1. Determine baseline (prior to initiation of any therapy) angiogenic response to cancer.**
- 2. Evaluate changes in tumor-associated angiogenesis as a biomarker of response following specific treatment modality (external beam irradiation, SU5416, or combination therapy).**
- 3. Correlate standard clinical/histopathologic parameters (i.e. primary tumor size, grade) to baseline and subsequent change in tumor-associated MVD in order to establish “angiogenic dependency” of human sarcomas.**

TECHNICAL APPROACH

Quantification of Tumor-Associated Microvessel Density

1. Rationale

SU5416 is a selective inhibitor of the Flk-1/KDR receptor tyrosine kinase expressed primarily on precursor and mature endothelial cells. The critical role of Flk-1 in tumor angiogenesis has been demonstrated in different studies where the receptor pathway has been disrupted utilizing dominant-negative strategies or neutralizing antibodies^{vii,viii}. SU5416 significantly decreases vascularity associated with neurogenic sarcoma xenografts in preclinical animal models^{ix}. Thus, the most likely candidate biomarker of response to SU5416 when administered to patients with soft tissue sarcomas is an alteration in tumor-associated MVD. We propose to assess MVD in patients receiving SU5416 alone or in combination with irradiation using reliable quantitative immunohistochemical techniques routinely used in our and other laboratories^{x,xi,xii}.

2. Sample Acquisition and Storage

a. Tissue Procurement/Specimen Criteria

Patients entered on this study must submit materials to the RTOG Tissue Bank. Ideally all specimens should be obtained via open surgical procedures. It is critical that special attention be given to specimen procurement. Samples should contain both viable tumor and attached adjacent stroma (Figure 1) to allow optimal determination of microvessel density (and study of peritumoral

angiogenic regulators outlined in other projects related to this protocol). Cytology preparations from fine needle aspirations cannot be used for this project, and core needle biopsies may not provide sufficient tissue to allow determination of MVD. All translational studies requiring formalin-fixed paraffin-embedded tissue will be performed on these same samples.

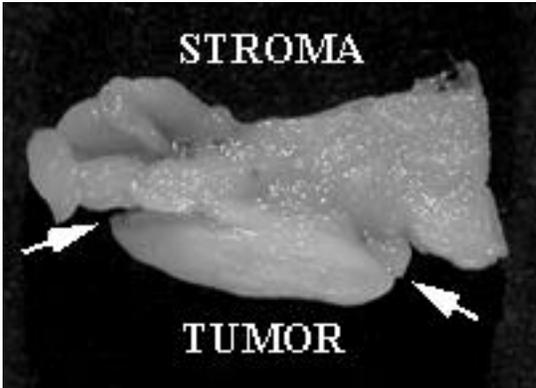


FIGURE 1: Example of specimen requested for these studies-contains non-necrotic sarcoma tissue with attached adjacent stromal tissue. Tumor-stromal interface indicated by arrows. Translational studies involving immunohistochemistry will be performed on sections obtained from specimens in this orientation within the paraffin block.

b. Tissue Fixation/Processing

It is recommended that upon specimen removal (at either initial biopsy or surgical resection), tissues be trimmed to a thickness of 1-3 mm to allow adequate fixation. It is critical that specimens be oriented to allow microtome-sectioning perpendicular to the tumor-stroma interface (see Figure 1) when processing occurs at participating institutions (sections should contain both tumor and adjacent stroma to allow optimal evaluation of peritumoral area and quantification of tumor-associated angiogenesis). Specimens should be immediately fixed in 10% buffered formalin for a period of 12-48 hours. Specimens may be processed into paraffin-embedded tissue blocks (using standard histopathology protocols) at the participating institution (preferred method) or shipped via overnight carrier to the RTOG Tissue Bank (address under section A.2.c) during the fixation process; if specimens are to be shipped during fixation, it is critical that containers be completely filled with formalin in order to avoid potential tissue drying.

c. Sample Labeling/Shipping

Specimens placed in formalin for central processing at the RTOG Tissue Bank should be packed securely to prevent fixative leakage and shipped via overnight carrier at room temperature. Please notify RTOG Tissue Bank by phone, fax, or e-mail, of intent to ship. Do not ship on Friday, Saturday, or Sunday. Paraffin-embedded tissue blocks may be stored at room temperature until shipment via either ground or overnight carrier.

The following must be provided to the RTOG Tissue Bank:

- 1) At least one (two samples are requested but only one is required) paraffin-embedded tissue block meeting the specimen criteria outlined in section A.2.a. Block must be clearly labeled with the pathology identification number that agrees with the pathology report.
- 2) Pathology report documenting that submitted block contain tumor.
- 3) A RTOG Pathology Submission Form must be included and must clearly state that it is being submitted for the RTOG Tissue Bank.

RTOG Pathology Submission Form
SURGICAL PATHOLOGY SPECIMEN

Acquisition Date: _____ Originating Institution: _____
Surgical Pathology #(s) from originating institution (if applicable): _____
Number of samples enclosed: _____

PATIENT IDENTIFICATION

RTOG Study #: _____ Case #: _____

SAMPLE INFORMATION (check and answer where appropriate)

Timeline: 1) initial biopsy (i.e. prior to receiving SU5416, chemotherapy or irradiation)
(check 2) surgical resection

Site: location of tumor/sample? _____

Therapy received prior to surgery: none (initial biopsy)
 irradiation
 chemotherapy
 SU5416

Samples enclosed: tumor-stroma interface (1-2 separate samples)
 perpendicular cross-section of open biopsy scar

CONTACT INFORMATION (person responsible for sample)

Name: _____ Ph #: _____ E-mail: _____

Materials should be sent to:

**LDS Hospital
Dept. of Pathology
E.M. Laboratory
8th Ave & C Street
Salt Lake City, UT 84143
(801) 408-5626
FAX (801) 408-5020
Ldafurne@ihc.com**

3. Laboratory Methods

a. Immunohistochemistry

Previously described standard immunoperoxidase techniques will be utilized for immunostaining. Briefly, five-micron sections will be cut from paraffin-embedded tissue blocks. Antigen retrieval, which has been shown to give optimal results for most antibodies

tested in our lab, will be performed on all slides to be immunostained for CD34 by boiling deparaffinized sections for ten minutes in citrate buffer (pH 6.0) using a 750 W microwave oven at low setting^{xiii}. Immunohistochemical staining will be performed in conjunction with an avidin-biotin-peroxidase kit (Vectastain Elite, Vector, Burlingame, CA). Specimens will undergo peroxidase blocking prior to overnight incubation at 4 °C with purified mouse anti-human CD34 [QBEnd/10] monoclonal antibody (BioGenex, San Ramon, CA; Cat. # MU236-UC). The sections will then be exposed to biotinylated secondary antibody (BioGenex Cat. # HK335-9M), an avidin-biotinylated complex and the chromagen diaminobenzidine (DAB). Tyramide signal amplification (NEN Life Science Products, Boston, MA) will be incorporated into this protocol to enhance chromagenic visualization for image analysis. Negative controls of test sections will be simultaneously prepared by omitting the secondary antibodies. Following completion of the immunohistochemical staining procedure, all sections will be counterstained with hematoxylin.

b. Microvessel Density Determination

In our laboratory, MVD is determined using systematic computer-assisted digital image analysis of non-contiguous microscopic fields in which vascular density is quantitated as percent endothelial-immunostained area present within 100 µM of the tumor-stroma interace. This methodology tends to improve reproducibility by compensating for inherent intratumoral variability when “vascular hot spots” are selectively evaluated at only high magnification.

Statistics

Microvessel densities are expected to be nonnormal, and, therefore, nonparametric testing is likely to be used. MVD associated with the baseline (initial) biopsy and resection specimen will be compared using ANOVA. The Kruskal-Wallis /Mann-Whitney test, and the Spearman rank correlation coefficient may be used to compare MVD at either time point to the levels of soluble angiogenic growth factors.

Personnel Involved in Sample Analysis

James C. Watson	Alice Zalatoris M.D.	laboratory technician
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Timeline for Sample Analysis

Sample analysis will be performed at quarterly intervals based on availability. Assays will commence when all samples from the same patient (initial biopsy, resection specimen) have been received in order to minimize the effect of inter-assay variation. All reagents, equipment, and technical support are currently available so that sample analysis may begin as soon as protocol is initiated.

EXPECTED RESULTS

MVD may be associated with aggressive disease (e.g. a high mitotic count and/or a large tumor bulk). Since SU5416 targets an endothelial specific receptor critical to angiogenesis regulation, one might hypothesize that tumors with greater initial MVD may be more dependent on neovascularization and thus realize a better response to this form of therapy (or in combination with classic chemo-irradiation). Pre- and post-treatment comparison should allow for detection of differences. In addition, patient's material from the high grade study can be compared to archived material from RTOG 95-14 with the variable being SU5416 administration.

PUBLICATION POLICY AND DATA OWNERSHIP

Publication of data will be considered as a joint publication by the principle investigators and RTOG and will be subject to RTOG publication guidelines. The results will be published as one (or more) article(s) in a peer-reviewed medical journal(s). The principal laboratory investigator is responsible for preparing the manuscript, and the co-authors and RTOG will receive copies of any intended communication in advance of publication for review.

¹ Weidner N, Semple JP, Welch WR, Folkman J. Tumor angiogenesis and metastasis - correlation in invasive breast carcinoma. *NEJM* 1991, **324**: 1-8.

² Weidner N, Semple JP, Welch WR, Folkman J. Tumor angiogenesis and metastasis - correlation in invasive breast carcinoma. *NEJM* 1991, **324**: 1-8.

³ Takebayashi Y, Akiyama S.I., Yamada K, Akiba S, Aikou T. Angiogenesis as an unfavorable prognostic factor in human colorectal carcinoma. *Cancer* 1996, **78**: 226-231.

⁴ Bochner BH, Cote RJ, Weidner, et al. Angiogenesis in bladder cancer: relationship between microvessel density and tumor prognosis. *J. Natl. Cancer Inst.* 1995, **87**: 1603-1612.

⁵ Saenz NC, Heslin MJ, Adsay V, et al. Neovascularity and clinical outcome in high-grade extremity soft tissue sarcomas. *Ann Surg Oncol* 1998, **5**:48-53.

⁶ Kawauchi S, Fukuda T, Tsuneyoshi M. Angiogenesis does not correlate with prognosis or expression of vascular endothelial growth factor in synovial sarcomas. *Oncol Rep* 1999, **6**:959-64.

⁷ Millauer B, Shawver LK, Plate KH, Risau W, Ullrich A. Glioblastoma growth inhibited *in vivo* by a dominant-negative Flk-1 mutant. *Nature* 1994, **367**: 576-579.

⁸ Rockwell P, Witte L, Hicklin D, et al. Antitumor activity of anti-Flk-1 monoclonal antibodies. *Proc Am Assoc Cancer Res* 1997, **38**: 266.

⁹ Angelov L, Sallhia B, Roncari L, et al. Inhibition of angiogenesis by blocking activation of the vascular endothelial growth factor receptor 2 leads to decreased growth of neurogenic sarcomas. *Cancer Res* 1999, **59**: 5536-5541.

- ¹⁰ Devinemi D, Klein-Szanto AJP, Gallo JM. Uptake of temozolomide in rat gliomamodel in the presence and absence of the angiogenesis inhibitor TNP-470. *Cancer Res. (Adv. In Brief)* 1996, **56**: 1983-1987.
- ¹¹ Ma J, Zhou-Li F, Klein-Szanto A, Gallo JM. Modulation of angiogenesis by human glioma xenograft models that differentially express vascular endothelial growth factor. *Clin. Exp. Metastasis* 1998, **16**: 559-568.
- ¹² Bolontrade MF, Stern MC, Binder RL, Zenklusen JC, Gimenez-Conti IB, Conti CJ. Angiogenesis is an early event in the development of chemically induced skin tumors. *Carcinogenesis* 1998, **19**: 2107-2113.
- ¹³ Shi S, Key ME, Kalra KL. Antigen retrieval in formalin-fixed, paraffin-embedded tissues: an enhancement method for immunohistochemical staining based on microwave oven heating of tissue sections. *J Histochem Cytochem* 1991, **39**: 741-748

APPENDIX VIII

Project 2 Tumor-Associated Pro-Angiogenic Regulators in Patients with Soft Tissue Sarcomas

**James C. Watson, M.D., Principal Investigator
Fox Chase Cancer Center**

Angiogenesis is not a passive process, but is driven by disturbances in the balance of positive and negative regulators. Appropriate factors must be expressed to initiate basement membrane degradation, endothelial cell proliferation and migration, and capillary tubule formation. Numerous angiogenic factors have been described, but vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) have emerged as the most likely candidates capable of promoting the angiogenic switch^{xiv}.

SPECIFIC AIMS

To quantify pro-angiogenic regulators associated with human adult soft tissue sarcomas both before and after treatment in order to accomplish the following:

4. Evaluate changes in tumor-associated endothelial growth factor receptors KDR, FLT-1, bFGFr) as a biomarker of response following specific treatment modality (external beam irradiation, SU5416, or combination therapy).
5. Evaluate changes in tumor-produced pro-angiogenic regulators (VEGF, bFGF) as a biomarker of response following specific treatment modality (external beam irradiation, SU5416, or combination therapy).

TECHNICAL APPROACH

Quantification of Pro-Angiogenic Regulators

4. Rationale

The best studied of the angiogenic regulators is VEGF. Active as both an endothelial cell-specific mitogen^{xv} and as a vascular permeability factor, it is produced by a large variety of human tumors^{xvi}. In tumors, VEGF synthesis is regulated by a number of factors including the environment (i.e. hypoxia, glucose levels, and nature of the host tissue), growth factors, oncogenes such as *ras*, and mutated tumor suppressor genes such as von Hippel Landau^{xvii}. Expression of VEGF by invasive tumors has been shown to correlate with vascularity, cellular proliferation and the ability to metastasize of several human cancers^{xviii,xix,xx}. We have reported quantitative immunohistochemical analysis of VEGF in human tumor xenograft models^{xxi}. In these systems, VEGF expression was directly associated with tumor aggressiveness, suggesting that it plays a critical role in progression to a more aggressive phenotype. Three high affinity endothelial-specific VEGF receptors have been identified that include KDR (kinase insert domain containing receptor)^{xxii,xxiii} and Flt-1 (fms-like tyrosine kinase)^{xxiv}. *In situ* hybridization has shown that KDR and Flt-1 are expressed exclusively on endothelial cells^{xxv,xxvi}. KDR is involved in VEGF-induced mitogenesis, but the role of Flt-1 is not clear^{xxvii}. Developmental studies have identified KDR on hemangioblasts, the precursor to hematopoietic and endothelial cells, as a biomarker of cells ultimately destined to become either vascular or lymphatic endothelium^{xxviii,xxix}. We have reported that the *in vitro* transformation of resting vascular endothelium to an angiogenic phenotype is associated with up-regulation of the VEGF-specific receptor *kdr* gene²⁵. In addition, studies on tumor angiogenesis have shown that expression of KDR on endothelial cells correlates with vascularity, metastasis, and proliferation of human colon cancer^{xxx}, the growth of human gliomas^{xxxi}, and correlates with human hepatic tumorigenesis^{xxxii}. These studies provide strong evidence that KDR is critical for the transformation to an angiogenic phenotype.

SU5416 is a selective inhibitor of the Flk-1/KDR receptor tyrosine kinase expressed primarily on precursor and mature endothelial cells. The critical role of Flk-1 in tumor angiogenesis has been demonstrated in different studies where the receptor pathway has been disrupted utilizing dominant-negative strategies or neutralizing antibodies^{xxxiii,xxxiv}. SU5416 significantly decreases vascularity associated with neurogenic sarcoma xenografts in preclinical animal models^{xxxv}.

Fibroblast growth factors (FGFs) make up a family of nine species showing a wide spectrum of biological activities that include regulation of angiogenesis, cell proliferation, and cell-to-cell adhesion^{xxxvi}. All of the family members are characterized by their strong affinity for heparin, and all are functional ligands for FGF receptors that have intrinsic tyrosine kinase activity. More direct evidence comes from the demonstration of inhibition of tumor growth in nude mice *in vivo* by neutralizing antibodies to FGF-2 (bFGF)^{xxxvii}. We have shown that angiogenic endothelium expresses mRNA for both bFGF and its receptor^{xxxviii}. Interestingly, VEGF and bFGF have been shown to synergize using *in vivo* angiogenesis assays^{xxxix}, indicating that they can serve complementary functions, consistent with their common association in tumors. Furthermore, antibody blockade of tumor-secreted VEGF or bFGF inhibits tumor growth^{xl, xli}. VEGF and bFGF act as major tumor-produced regulators capable of promoting the angiogenic switch^{xlii}. These growth factors and their respective receptors may serve as likely candidate biomarkers of response to SU5416 when administered to patients with soft tissue sarcomas. We propose to assess VEGF, bFGF, KDR, FLT-1 and bFGF in tissue pre-treatment (baseline) and post-treatment in patients given SU5416 in a combined modality schema or in patients treated with radiation alone. This will be accomplished by using reliable quantitative immunohistochemical techniques routinely employed in our and other laboratories^{xliii, xliv, xlv, xlvi}.

5. Sample Acquisition and Storage

a. Tissue Procurement/Specimen Criteria

Patients entered on this study must submit materials to the RTOG Tissue Bank. Ideally, all specimens should be obtained via open surgical procedures. It is critical that special attention be given to specimen procurement. Samples should contain both viable tumor and adjacent (attached) stroma to allow optimal determination of proposed peritumoral biomarkers. All translational studies using formalin-fixed paraffin-embedded tissue will be performed on the same specimens (see Appendix VII)—duplicate specimens for each study are not necessary.

b. Tissue Fixation/Processing

All translational studies using formalin-fixed paraffin-embedded tissue will be performed on the same specimens. See Appendix VII for instructions on tissue fixation and processing.

c. Sample Labeling/Shipping

All translational studies using formalin-fixed paraffin-embedded tissue will be performed on the same specimens. See Appendix VII for instructions on sample labeling and shipping.

6. Laboratory Methods

c. Immunohistochemistry

Previously described standard immunoperoxidase techniques will be utilized for immunostaining. Briefly, five-micron sections will be cut from paraffin-embedded tissue blocks. Antigen retrieval, which has been shown to give optimal results for most antibodies tested in our lab, will be performed on all slides to be immunostained for VEGF and bFGF

by boiling deparaffinized sections for ten minutes in citrate buffer (pH 6.0) using a 750 W microwave oven at low setting^{xlvi}. Immunohistochemical staining will be performed in conjunction with an avidin-biotin-peroxidase kit (Vectastain Elite, Vector, Burlingame, CA). Specimens will undergo peroxidase blocking prior to overnight incubation at 4 °C with either purified rabbit anti-VEGF [147] polyclonal antibody (Santa Cruz Cat. # sc-507), goat anti- FGF-2 [147] polyclonal antibody (Santa Cruz Cat. # sc-79), mouse anti-human Flk-1 [A-1] monoclonal antibody (Santa Cruz Cat. # sc-6251), rabbit anti-Flt-1 [H-225] polyclonal antibody (Santa Cruz Cat. # sc-9029), or mouse anti-human Bek [C-8] monoclonal antibody (anti-FGFR-2; Santa Cruz Cat. # sc-6930). The sections will then be exposed to appropriate biotinylated secondary antibody, an avidin-biotinylated complex and the chromagen diaminobenzidine (DAB). Negative controls of test sections will be simultaneously prepared by omitting the secondary antibodies. Following completion of the immunohistochemical staining procedure, all sections will be counterstained with hematoxylin.

c. Quantification of Immunostain

Intensity of staining for VEGF, bFGF, and bFGFr (each with less localization than the endothelial specific KDR and FLT-1) will be graded on a scale of 0-3+, with 0 representing no detectable stain and 3+ representing the strongest stain. All specimens containing each type of histology (i.e. normal epithelium, pre-invasive lesion or tumor) will be randomly evaluated on three separate occasions without knowledge of treatment, the expression of the other angiogenic factors or MVD. In our previous experience, concordance has been greater than 90% for repeated measures utilizing this method^{3,xlviii}. The median value of each type of histology present on individual slides will then be selected as the measure for that particular histology to be used for further calculations. Selected markers will be more precisely quantitated by computed-assisted image analysis (Roche Pathology Workstation, Roche Image Analysis Systems/Autocyte, Elon College, NC). Quantitation of immunostain will be expressed as a ratio of the respective integrated optical densities

Computer-assisted digital image analysis of non-contiguous microscopic fields within 100 µM of the tumor-stroma interface will be used to quantitate the endothelial specific VEGF receptors (KDR and FLT-1). This methodology will allow quantitation of these receptors based both on stain intensity and as percent area immunostained.

Statistics

Levels of pro-angiogenic regulators are expected to be nonnormal, and, therefore, nonparametric testing is likely to be used. Growth factor (and receptor) levels associated with the baseline (initial) biopsy and resection specimen will be compared using ANOVA. The Kruskal-Wallis /Mann-Whitney test, and the Spearman rank correlation coefficient may be used to compare tumor-associated pro-angiogenic regulator levels to tumor associated MVD (Project 1) and levels of soluble angiogenic growth factors (Project 4).

Personnel Involved in Sample Analysis

James C. Watson	Alice Zalatoris M.D.	laboratory technician
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Timeline for Sample Analysis

Sample analysis will be performed at quarterly intervals based on availability. Assays will commence when all samples from the same patient (initial biopsy, resection specimen) have been received in order to minimize the effect of inter-assay variation. All reagents, equipment, and technical support are currently available so that sample analysis may begin as soon as protocol is initiated.

EXPECTED RESULTS

High levels of pro-angiogenic regulators may be associated with aggressive disease (e.g. a high mitotic count and/or a large tumor bulk). Correspondingly, tumor-associated MVD should correlate to tumor-produced proangiogenic regulators. Since SU5416 targets KDR, one might hypothesize that decreases in this receptor should occur following treatment with this agent. Comparison of baseline to resection specimens as per protocol design should allow determination of effect of different treatment modalities (SU5416, chemo-irradiation or radiation alone) on tumor-associated pro-angiogenic regulators. Comparison of high-grade tumor specimens to archived RTOG 95-14 tissue specimens should allow dissection of drug from radiation effect.

PUBLICATION POLICY AND DATA OWNERSHIP

Publication of data will be considered as a joint publication by the principle investigators and RTOG and will be subject to RTOG publication guidelines. The results will be published as one (or more) article(s) in a peer-reviewed medical journal(s). The principal laboratory investigator is responsible for preparing the manuscript, and the co-authors (listed on the cover page) and RTOG will receive copies of any intended communication in advance of publication for review.

APPENDIX IX

Project 3 Tumor Cell Proliferation and Apoptosis as Surrogate Markers of Treatment Response to SU5416

**James C. Watson, M.D., Principal Investigator
Fox Chase Cancer Center**

Dysregulation of apoptosis may contribute to tumor formation and malignant progression by allowing the accumulation of proliferating cell populations and obstructing the elimination of cells with genetic abnormalities conferring enhanced malignant potential^{xlix,l}. Evaluation of both proliferation and apoptotic indices within a neoplastic lesion has been utilized to evaluate the efficacy in cancer therapeutics^{li,lii,liii}. To date, no studies have been reported evaluating changes in human soft tissue sarcoma cell proliferation or apoptosis associated with anti-angiogenic therapies.

SPECIFIC AIM

To quantify tumor cell proliferation and apoptosis within human adult soft tissue sarcomas both before and after treatment in order to evaluate whether alterations can serve as a biomarker of response following specific treatment modality (external beam irradiation, SU5416, or combination therapy).

TECHNICAL APPROACH

Quantification of Tumor Cell Proliferation and Apoptosis

7. Rationale

SU5416 significantly inhibits tumor-associated angiogenesis resulting in reduction of human neurogenic sarcoma xenograft growth in mouse models^{liv}. This effect is associated with a reduction in tumor cell proliferation and an increase in tumor cell apoptosis. Immunohistochemical quantitation of the cell cycle antigen Ki-67 in soft tissue sarcomas correlates with most standard clinicopathologic parameters, including tumor size, malignancy grade, necrosis, vascular invasion, S-phase fraction, and metastasis^{lv}. The TUNEL technique entails a non-isotopic in situ end-labeling of fragmented DNA, characteristically produced by non-random nucleosomal fragmentation during programmed cell death (Buja).

8. Sample Acquisition and Storage

d. Tissue Procurement/Specimen Criteria

Patients entered on this study must submit materials to the RTOG Tissue Bank. Ideally, all specimens should be obtained via open surgical procedures. It is critical that special attention be given to specimen procurement. Samples should contain both viable tumor and adjacent (attached) stroma to allow optimal determination of proposed peritumoral biomarkers. Relevant paraffin-embedded core biopsy tumor samples (obtained at initial diagnosis in patients who do not initially undergo open surgical biopsy) may also be used provided they contain viable tumor cells. All

translational studies using formalin-fixed paraffin-embedded tissue will be performed on the same specimens (see Appendix VII)—duplicate specimens for each study are not necessary.

e. Tissue Fixation/Processing

All translational studies using formalin-fixed paraffin-embedded tissue will be performed on the same specimens. See Appendix VII for instructions on tissue fixation and processing.

f. Sample Labeling/Shipping

All translational studies using formalin-fixed paraffin-embedded tissue will be performed on the same specimens. See Appendix VII for instructions on sample labeling and shipping.

9. Laboratory Methods

d. Determination of Cell Proliferation

1. Immunohistochemistry: Immunohistochemistry will be used to determine the percentage of proliferating cells in each tumor specimen by identifying the nuclear antigen Ki-67 using Mib-1 antibody^{lvii}. Previously described standard immunoperoxidase techniques will be utilized for immunostaining. Briefly, five-micron sections will be cut from paraffin-embedded tissue blocks. Antigen retrieval, which has been shown to give optimal results for most antibodies tested in our lab, will be performed on all slides using trypsin digestion at 37°C for 10 minutes. Immunohistochemical staining will be performed in conjunction with an avidin-biotin-peroxidase kit (Vectastain Elite, Vector, Burlingame, CA). Specimens will undergo peroxidase blocking prior to overnight incubation at 4°C with purified mouse anti-Ki-67 [Mib-1] (Zymed Laboratories, San Francisco, CA; Cat. #08-0192). The sections will then be exposed to biotinylated secondary antibody (BioGenex Cat. #HK335-9M), an avidin-biotinylated complex and the chromagen diaminobenzidine (DAB). Sections from small intestine will be used as positive control. Negative controls of test sections will be simultaneously prepared by omitting the secondary antibodies. Following completion of the immunohistochemical staining procedure, all sections will be counterstained with hematoxylin.

2. Quantification of Immunostain: The percentage of Mib-1 positive cancer cells will be determined and will be processed as a classical labeling index (% of stained nuclei/total number of nuclei). A minimum number of 1,000 cells per lesion will be counted when available. For small lesions all cells will be counted.

b. Apoptosis Analysis

1. TUNEL Assay: In situ detection of apoptosis by use of the terminal deoxynucleotidyl transferase (TdT) dUTP nick-end labeling (TUNEL) technique will be performed as described previously^{lviii} using the ApopTag® in situ apoptosis detection kit (ApopTag, Oncor, Gaithersburg, MD), which identifies cells with internucleosomal fragmentation of DNA. Briefly, deparaffinized and rehydrated formalin-fixed tissue sections will be incubated with proteinase K (20 mg/mL) at room temperature for 15 minutes. Endogenous peroxidase will be inactivated by 3% hydrogen peroxide. Tissue sections were then subjected to the ApopTag reaction. Antidigoxigenin-peroxidase solutions will be added and diaminobenzidine will be used to detect the labeled nuclei. For negative controls, deionized water will be used instead of TdT. Inflamed human tonsil will serve as a positive control.

2. Quantification of Apoptosis: The percentage of positive cancer cells will be used to determine the apoptotic index (% of stained nuclei/total number of nuclei). A minimum number of 1,000 cells per lesion will be counted when available. For small lesions all cells will be counted.

Statistics

Tumor cell proliferative and apoptotic indices are expected to be nonnormal, and, therefore, nonparametric testing is likely to be used. Baseline (initial) biopsy and resection specimen indices will be compared using ANOVA. The Kruskal-Wallis /Mann-Whitney test, and the Spearman rank correlation coefficient may be used to compare indices to tumor associated MVD (Project 1) and levels of soluble angiogenic growth factors (Project 4).

Personnel Involved in Sample Analysis

	Alice Zalatoris	laboratory technician
James C. Watson	M.D.	

Timeline for Sample Analysis

Sample analysis will be performed at quarterly intervals based on availability. Assays will commence when all samples from the same patient (initial biopsy, resection specimen) have been received in order to minimize the effect of inter-assay variation. All reagents, equipment, and technical support are currently available so that sample analysis may begin as soon as protocol is initiated.

EXPECTED RESULTS

High tumor cell proliferative indices may be associated with aggressive disease (e.g. a high mitotic count and/or a large tumor bulk). Correspondingly, either marked reduction in tumor cell proliferative index or an increase in the apoptotic index may correlate to treatment effect. Since SU5416 targets KDR, one might hypothesize that inhibition of angiogenesis should result in an increase in apoptotic index at time of resection (and following treatment) compared to baseline (initial biopsy). Comparison of baseline to resection specimens should allow determination of effect of different treatment modalities (SU5416, chemo-irradiation). Comparison of high-grade tumor specimens to archived RTOG 95-14 tissue specimens should allow dissection of drug and radiation effect from SU5416 effect.

PUBLICATION POLICY AND DATA OWNERSHIP

Publication of data will be considered as a joint publication by the principle investigators and RTOG and will be subject to RTOG publication guidelines. The results will be published as one (or more) article(s) in a peer-reviewed medical journal(s). The principal laboratory investigator is responsible for preparing the manuscript, and the co-authors (listed on the cover page) and RTOG will receive copies of any intended communication in advance of publication for review.

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APPENDIX X

Project 4 Soluble Angiogenic Growth Factors as Surrogate Markers of Treatment Response in Patients with Soft Tissue Sarcomas

**James C. Watson, M.D., Principal Investigator
Fox Chase Cancer Center**

Tumor-induced angiogenesis is regulated by many different growth factors and growth factor receptors. Among several identified soluble peptides with angiogenic properties, vascular endothelial growth factor (VEGF) and basis fibroblast growth factor (bFGF) have been identified as powerful promoters of tumor-induced angiogenesis^{lxviii,lix}. Both growth factors can be detected in human body fluids by specific enzyme-linked immunosorbent assays^{lx,xli}, and elevated serum concentrations of each are detectable in patients with various types of tumor, including soft tissue sarcomas^{lxii,lxiii,lxiv}. To date, no studies have been reported evaluating treatment-related alterations of these soluble angiogenic growth factors in patients with soft tissue sarcomas.

SPECIFIC AIMS

To serially quantify levels of soluble VEGF and bFGF during treatment of adult patients with soft tissue sarcomas in order to accomplish the following:

1. Evaluate changes in soluble angiogenic growth factor levels as a biomarker of response following specific treatment modality (external beam irradiation, SU5416, or combination chemotherapy).
2. Correlate standard clinical/histopathologic parameters (i.e. tumor mass, grade) to baseline and subsequent changes in level of soluble angiogenic growth factors.

TECHNICAL APPROACH

Quantification of Soluble Angiogenic Growth Factors

10. Rationale

SU5416 is a selective inhibitor of the VEGF tyrosine kinase receptor KDR expressed primarily on precursor and mature endothelial cells. VEGF is secreted by various human cancer cells and plays a key role in cancer angiogenesis and metastasis^{lxv}. Alternatively, some cancers are less dependent on VEGF and other proangiogenic regulators such as bFGF are believed to be more critical in promoting an angiogenic response^{lxvi}.

Circulating angiogenic factors can be measured from either serum or plasma. Practically all circulating VEGF is carried within the platelets and the leukocytes¹⁰. Since VEGF measured from serum has its origin in the blood cells, hemolysis or platelet activation may result in liberation of VEGF into serum^{lxvii}. However, platelet-derived VEGF is suggested to reflect the biology of cancer cells due to implications that platelet-tumor interactions may occur during metastasis^{lxviii}. Alternatively, the use of the whole blood as the starting material may be safer than using the serum

when the levels of circulating VEGF are being assessed, because sample handling is of less importance since plasma can be isolated after controlled lysis of blood cells at a central facility. Plasma VEGF levels have been found to be higher in cancer patients as compared with healthy controls when assessed per one platelet or per one white blood cell.

11. Sample Acquisition and Storage

g. Sample Criteria and Processing Requirements

Patients entered on this study must submit blood samples for evaluation of soluble angiogenic growth factors. Patient consent form on record at RTOG headquarters should give the authority and responsibility to comply with this request.

Blood samples may be obtained using either peripheral venipuncture or via central venous catheters (using appropriate technique to discard initial aspirate used to flush lines). Blood should be collected into two distinct test tubes: 1) ordinary serum (clotting) tube and 2) tubes containing sodium citrate (anticoagulant). At least one 5 ml test tube of each type should provide adequate volumes for evaluation (minimum of 1ml of serum and 2 ml of whole blood are required). After procurement, samples may be incubated at +4°C up to 1-4 hrs prior to serum processing (which must be performed at participating institutions prior to shipment). In order to process for shipment, serum (clot) tubes should be centrifuged at 2000g for 10 minutes at +4°C and then serum should be transferred to a separate sterile tube prior to storage at -20°C or colder until shipment. Tubes containing whole blood should be stored at +4°C until shipment. Shipment to the processing laboratory at Fox Chase Cancer Center (address below) should be made within 24 hours of each procurement.

h. Timeline for Sample Acquisition

Serum and whole blood are to be collected from patients at four different time points: 1) after initial diagnosis has been made prior to receiving any form of therapy (includes cytotoxic chemotherapy, irradiation, or SU5416), 2) mid-treatment (1-2 weeks prior to resection) after having received at least four doses of SU5416 [two separate samples: (1) prior to receiving regular scheduled SU5416 dose, and (2) within one hour of completion of SU5416 infusion]. Note: In the RT alone group only need one mid-treatment sample. 3) morning of resection (ideally this sample should be drawn prior to operative procedure since surgery is known to influence VEGF levels^{lxix}), and 4) at the final post-resection follow-up (usually 3-6 weeks following resection) .

i. Sample Labeling/Shipping

Samples should be shipped to the central processing laboratory at Fox Chase Cancer Center (address below) with 24 hours following EACH procurement. Blood and serum samples should be packed securely in appropriate insulated containers with cold-packs (whole blood should not be frozen) to prevent damage/leakage or heat denaturation and shipped directly to the RTOG Tissue Bank via overnight carrier. At least one sample (test tube) of EACH of the following: 1) whole blood in sodium citrate (≥2 ml) and 2) isolated serum (≥1 ml). Samples must be clearly labeled with the patient's name and date of procurement. The following is an example of the Pathology Submission Form that must be accurately filled out and included with blood/serum samples being submitted to the central processing laboratory at Fox Chase Cancer Center.

RTOG Pathology Submission Form
BLOOD/SERUM SPECIMENS

Acquisition Date: _____ Originating Institution: _____

PATIENT IDENTIFICATION

RTOG Study #: _____ Case #: _____

SAMPLE INFORMATION (check and answer where appropriate)

- Timeline: 1) prior to therapy (i.e. no SU5416, chemotherapy or irradiation)
(check -requires two samples - "baseline" (initial) and "1st peak" (within one hour of SU5416 administration)—PLEASE LABEL ACCORDINGLY
- 2) during SU5416 administration (after at least four doses of SU5416 administered)
-requires two samples - "trough" (initial) and "2nd peak" (within one hour of regular SU5416 administration)—PLEASE LABEL ACCORDINGLY
- Number of SU5416 doses received prior to "trough" sample? _____
- 3) at time of resection
-Was sample obtained prior to actual surgery (i.e. incision)? _____
- 4) at follow up
- Number of days post-surgical procedure? _____

- Source: peripheral venipuncture
(check central venous line (after initial aspirate appropriately discarded)
 arterial

Samples enclosed: serum (≥ 5 ml) whole blood (≥ 5 ml)

CONTACT INFORMATION (person responsible for sample)

Name: _____ Ph #: _____ E-mail: _____

Materials should be sent to:

**Fox Chase Cancer Center
Dept. of Surgical Oncology
Room c403
7701 Burholme Avenue
Philadelphia, PA 19111
(215) 728-7094
AC_Zalatoris@fccc.edu
JC_Watson@fccc.edu**

j. Sample Storage

Plasma will be obtained from whole blood samples at the central processing laboratory at Fox Chase Cancer Center. Serum and plasma will be divided into small aliquots (100-500 μ l) and stored at the central processing laboratory at Fox Chase Cancer Center at -70°C until further processing.

12. Laboratory Methods - Enzyme-linked immunosorbent assay (ELISA)

e. VEGF analysis

Serum and whole blood VEGF levels are determined as serum VEGF immunoreactivity using a quantitative sandwich enzyme immunoassay technique (Quantikine® Human VEGF Immunoassay, R&D Systems, Minneapolis, MN). The system uses a solid phase monoclonal and an enzyme-linked polyclonal antibody raised against recombinant human VEGF. For each analysis, 100 µl of serum is used. All analyses and calibrations are carried out in duplicate. The calibrations on each microtiter plate include recombinant human VEGF standards. Optical densities are determined using a microtiter plate reader at 450 nm. The blank is subtracted from the duplicate readings for each standard and sample. A standard curve is created by plotting the logarithm of the mean absorbance of each standard versus the logarithm of the VEGF concentration. Concentrations are reported as pg/ml. All samples of the same patient will be analyzed on the same ELISA plate to minimize inter-assay variation.

b. bFGF Analysis

Serum and blood bFGF concentrations are determined as serum bFGF immunoreactivity using a quantitative sandwich enzyme immunoassay technique (Quantikine® High Sensitivity Human FGF basic Immunoassay; R&D Systems) as described above. The system uses a solid phase monoclonal and an enzyme-linked polyclonal antibody against recombinant human bFGF. The analysis is performed essentially as the VEGF immunoassay. Optical densities are determined at 490 nm.

Statistics

The growth factor distributions are expected to be nonnormal, and, therefore, nonparametric testing is likely to be used. Wilcoxon's signed rank-test will be used for paired comparisons of growth factor concentrations obtained at different time points. The Kruskal-Wallis/Mann-Whitney tests will be used for correlating growth factor levels with tumor status and histopathologic parameters.

C. Personnel Involved in Sample Analysis

Alice Zalatoris	laboratory technician
James C. Watson	M.D.

D. Timeline for Sample Analysis

Sample analysis will be performed based on sample availability. Assays will commence when all (four) samples from a single patient have been received in order to minimize the effect of inter-assay variation. (It is likely that assays will be performed every three months to maximize usage of ELISA assay). Results should be available within 30 days of commencing assay (estimated 3-4 assays/year).

EXPECTED RESULTS

Baseline (untreated) serum/blood VEGF and bFGF levels are elevated in 77.6 and 72.9% of patients with soft tissue sarcoma, respectively. It is anticipated that a significant correlation will exist with baseline levels and standard clinical/histopathologic parameters such as tumor mass and histologic grade. High serum angiogenic growth factor levels may be associated with poor prognosis, but the relatively small number of patients (estimated n=36) and the relatively short follow-up time available may not allow to study fully the prognostic value of these serum factors in the context of the present analysis. To date, no studies have been reported evaluating treatment-related alterations of soluble angiogenic growth factor levels in patients with soft tissue sarcomas. We hypothesize that initially elevated VEGF and bFGF levels should decrease in response to therapy that diminishes tumor mass. A detectable difference may be reflective of the anti-angiogenic response induced by SU5416 particularly in those patients receiving RT alone vs. RT and SU5416. Markedly elevated soluble growth factor levels that do not decrease following treatment may be associated with aggressive disease (e.g. a high mitotic count and/or a large tumor bulk).

PUBLICATION POLICY AND DATA OWNERSHIP

Publication of data will be considered as a joint publication by the investigators and RTOG and will be subject to RTOG publication guidelines. The results will be published as one (or more) article(s) in a peer-reviewed medical journal(s). The principal investigator is responsible for preparing the manuscript, and the co-authors (listed on the cover page) and RTOG will receive copies of any intended communication in advance of publication for review.

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APPENDIX XI

Specimens Necessary for RTOG Correlative/Translational Studies

Evaluating Treatment Responses to SU5416 in Patients with Low/Intermediate Grade Soft Tis

Sample	Priority	Purpose and Proposed Techniques	Quantity	Pre-Treatment ¹	OnTreatment/ Pre-Resection ²	Post-Treat At Rese
				Baseline	1-2 wks prior to resection	Day (resect)
Surgical Pathology:Tumor-stroma interface ³	1	Immunohistochemistry ⁴ ,TUNEL assay ⁵	Paraffin-embedded Tissue Block ⁶	X ⁷	NA	X
Blood ⁸ - peripheral	2	ELISA ⁹	Serum =1 ml Whole blood =2ml	X ¹⁰	X ¹¹	X

Surgical Pathology: Biopsy scar ¹²	3	Immunohistochemistry ¹³	Paraffin- embedded Tissue Block ¹⁴	NA	NA	X
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¹All samples obtained with tumor *in situ* prior to initiating treatment.

² Following administration of at least four doses of SU5416; blood sample should be drawn at time of regularly scheduled SU5416 administration.

³Specimen must include interface between viable tumor and stroma in order to adequately evaluate proposed biomarkers; specimens should be fixed in formalin x 16-48 hrs then paraffin-embedded.

⁴ Immunohistochemistry to assess microvessel density (CD34) and angiogenic regulators (VEGF, KDR, FLT-1); tumor/endothelial cell proliferation evaluation (Ki67) – requires viable tumor-stroma interface.

⁵TUNEL assay to assess apoptosis in tumor and endothelial cells – requires viable tumor-stroma interface.

⁶Formalin-fixed tissue may be processed into paraffin-embedded tissue block at either participating institution or at RTOG Tissue Bank. Tissue blocks should be adequate for preparation of 12 unstained slides. The slides will be done at RTOG tumor bank.

⁷A pre-treatment biopsy should ideally be an open biopsy or can be multiple core biopsies done under CT guidance to provide tumor/stromal interface for baseline examination; core biopsy may not allow adequate assessment of peritumoral angiogenesis (MVD and endothelial specific receptors - KDR, FLT-1, bFGFr).

⁸Blood samples require processing (serum separation) at participating institution. See Appendix Project 4 for blood sample processing.

⁹Enzyme-linked immunosolvent assay to quantitate circulating angiogenic growth factors (VEGF, bFGF).

¹⁰Blood samples drawn prior to 1st drug treatment.

¹¹Blood samples drawn at two separate time points with regards to medication infusion: 1) prior to any medication (labeled as “trough” sample), and 2) within one-hour of the end of infusion of SU5416 (labeled as "peak" sample).

¹²Full thickness perpendicular cross-section of prior open biopsy scar (if applicable); should contain all layers of epidermis/dermis and immediately underlying stromal tissue.

¹³Immunohistochemistry to assess microvessel density (CD34) and angiogenic regulators (VEGF, KDR, FLT-1) to evaluate for SU5416 effect on wound healing.

¹⁴Formalin-fixed tissue may be processed into paraffin-embedded tissue block at either participating institution or at RTOG Tissue Bank. Tissue blocks should be adequate for at least 8 unstained slides. Slides will be prepared at RTOG Tumor Bank.

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APPENDIX XII

RTOG Study #: _____ Case #: _____
Date: _____

Pre-contrast imaging

- 1) Location of skin markers
 - Proximal marker: _____ cm to the (anatomic landmark)
 - Distal marker: _____ cm to the (anatomic landmark)
- 2) Long axis T1-weighted sequence
 - _____ Sagittal or _____ Coronal
 - _____ Field of view:
 - _____ Slice thickness:
 - _____ Interslice gap
 - _____ Matrix
 - _____ Slice number
- 3) Axial T1-weighted sequence
 - Coverage: From (anatomic landmark) to (anatomic landmark)
 - Field of view: _____
 - Slice thickness _____
 - Interslice gap _____
 - Matrix _____
 - Slice number _____
- 4) Axial FSE T2-weighted sequence
 - Coverage: From (anatomic landmark) to (anatomic landmark)
 - Field of view: _____
 - Slice thickness: _____
 - Interslice gap _____
 - Matrix _____
 - Slice number _____

Dynamic MR Imaging

- 1) I.V. cannula: ___20 gauge or ___22 gauge
- 2) Rate of infusion: _____
- 3) Gadopentetate dimeglumine dose: _____
- 4) Location of axial plane: _____ cm from (anatomic landmark)
- 5) T1-weighted gradient echo sequence
 - TR: _____ TE: _____ TI: _____
 - Field of view: _____
 - Slice thickness: _____
 - Interslice gap _____
 - Matrix _____

Post-contrast MR imaging

Long axis and axial T1-weighted imaging parameters must be identical to pre contrast T1-weighted images.