A PHASE I/II STUDY OF NEOADJUVANT CHEMOTHERAPY, ANGIOGENESIS INHIBITOR SU5416 (NSC #696819; A TK INHIBITOR ANTI-ANGIOGENESIS COMPOUND), AND RADIATION THERAPY IN THE MANAGEMENT OF HIGH-RISK, HIGH-GRADE, SOFT TISSUE SARCOMAS OF THE EXTREMITIES AND BODY WALL

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INDEX

Schema

Eligibility Check

1.0 Introduction

2.0 Objectives

3.0 Patient Selection

4.0 Pretreatment Evaluation

5.0 Registration Procedures

6.0 Radiation Therapy

7.0 Drug Therapy

8.0 Surgery

9.0 Other Therapy

10.0 Pathology

11.0 Patient Assessments

12.0 Data Collection

13.0 Statistical Considerations

References

Appendix I - Sample Consent Form
Appendix II - Karnofsky Performance Status
Appendix III - Staging Systems
  A - AJCC
  B - Enneking System
Appendix IV - Toxicity Criteria
Appendix V - Adverse Reaction Reporting Guidelines
Appendix VI - SPECT RBC Perfusion Scan Data Form
Appendix VII - Translational Research Project 1
Appendix VIII - Translational Research Project 2
Appendix IX - Translational Research Project 3
Appendix X - Translational Research Project 4
Appendix XI - Tissue/Blood Procurement Guidelines
Appendix XII - Response Evaluation Criteria in Solid Tumors (RECIST) Quick Reference
Appendix XIII - MR Imaging Data Form
Appendix XIV - Filgrastim (G-CSF) Drug Request Form
Appendix XV - Returned Medication (G-CSF) Packing Slip
RADIATION THERAPY ONCOLOGY GROUP
RTOG S-0121

A PHASE I/II STUDY OF NEOADJUVANT CHEMOTHERAPY, ANGIogenesis INHIBITOR SU5416 (NSC #696819; a TK INHIBITOR ANTI-ANGIOGENESIS COMPOUND), AND RADIATION THERAPY IN THE MANAGEMENT OF HIGH-RISK, HIGH-GRADE, SOFT TISSUE SARCOMAS OF THE EXTREMITIES AND BODY WALL

SCHEMA

<table>
<thead>
<tr>
<th>Tumor Location</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lower extremity (including hip)</td>
<td>Neoadjuvant chemotherapy/SU5416 x 3 cycles (progressive dose increase in phase I); followed by surgical resection and an additional 3 cycles of chemotherapy/SU5416 +/- RT Boost</td>
</tr>
<tr>
<td>Upper extremity (including shoulder)</td>
<td>See below and sections 7.1.1.7 and 13.3</td>
</tr>
<tr>
<td>Body wall</td>
<td></td>
</tr>
</tbody>
</table>

**Phase I SU5416 Doses**
- Arm 1: 85 mg/m²
- Arm 2: 110 mg/m²
- Arm 3: 145 mg/m²

**Phase II SU5416 Dose**
Arm 4: To be determined by phase I

**Neoadjuvant Chemotherapy (MAID)**
- Mesna 2500 mg/m²/day as a continuous intravenous infusion via a peripheral line, Days 1-4 (Optional: the same dose of Mesna can be given over 12 hours on day 4 only)
- Doxorubicin 20 mg/m²/day as a continuous intravenous infusion via a central line, Days 1-3
- Ifosfamide 2500 mg/m²/day as a continuous intravenous infusion, Days 1-3
- DTIC 225 mg/m²/day as a continuous intravenous infusion via a central line, Days 1-3
- G-CSF 5 mcg/kg/day administered as a subcutaneous injection starting on day 5 (24 hours after completion of the administration of the chemotherapy) and continuing daily until white blood cell count recovers (post radir granulocyte or ANC count of >10,000)

Repeat every 3 weeks x 3 cycles preoperatively, then again beginning 3-5 weeks after the resection for 3 additional cycles. See Section 8.2.1 for timing of surgery.

**SU5416 Angiogenesis Inhibitor**
SU5416 at 85mg/m² in the first 6 patients (See Section 7.1.1.7 for clinical design and toxicity criteria for 30% dose escalation); Sugen 5416 at 110 mg/m² for 6 patients; and then Sugen 5416 at 145 mg/m²/day as a continuous intravenous infusion via a central or peripheral line twice weekly on Mondays and Thursdays or Tuesdays and Fridays of each week throughout pre-operative chemotherapy and radiation therapy (and as a single agent following the completion of chemotherapy and radiation therapy per Section 7.1.1.6) stopping at least 2 days and no more than 5 days prior to surgery; and twice weekly during any post-operative chemotherapy and/or radiation therapy.

**Pre-operative Radiation Therapy**
Starting 3 days after each of the first 2 cycles of pre-operative chemotherapy, 2 cycles of 22 Gy (2 Gy x 11 fractions) will be given by external beam over 15 days, for a total dose of 44 Gy.

**Post-operative Radiation Therapy (only for patients with positive margins)**
Starting 14 days after surgery (assuming wound healing is good) 16 Gy (2 Gy x 8 fractions) will be given by external beam.

**Eligibility:** (See Section 3.0 for details)
- Histologically confirmed, locally confined, soft tissue sarcoma, Grade 2 or 3 on scale of 1-3 or Grades 3 or 4 on a scale of 1-4, ≥ 8 cm in maximum diameter by MRI or CT; Stage IIC and III (AJCC, 1998).
- Sarcoma located on upper (includes shoulder) or lower (includes hip) extremities or on body wall
- Zubrod ≤ 1
- WBC ≥ 4,000 or ANC ≥ 1800, platelets ≥ 150,000, bilirubin ≤ 1.5, creatinine ≤ 1.5, AST and ALT ≤ 1.5 x ULN, PT, PTT < 1.25 times normal (prior to coumadin), Fibrin Split products < 2 x normal/ Fibrinogen > 200 mg/dL
- Normal heart function (EF ≥ 50%); Patients with a history of a bypass surgery may only be enrolled if the surgery occurred at least one year prior to enrollment and after consultation with a cardiologist to determine stability of disease.
- No evidence of metastases; no concurrent malignancies or prior malignancies within preceding five years
- No prior chemotherapy, radiation, or biotherapy
- 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 history of a bleeding or clotting diathesis
- No severe peripheral vascular disease
- No contraindications to limb-salvage surgery
- No known hypersensitivity to E. coli derived proteins
- No concomitant uncontrolled medical or psychiatric disorders or any condition which compromises the patient’s ability to give informed consent or to complete this study as judged by the investigator
- Patients must not be pregnant or breastfeeding, and both genders must practice suitable contraception throughout the study.
- Treatment must begin within two weeks after registration.
- Patient must sign a study-specific consent form prior to registration.

**Required Sample Size:**

6-18 (phase I component)

62 (phase II component)

74 (estimated accrual to entire study)
1. Is the malignancy a primary or recurrent (after surgery only) soft tissue sarcoma? (Y)
2. What is the AJCC Stage (Appendix III)? (IIC/III)
3. Was histologic confirmation (biopsy) done within 2 months prior to registration?
4. What is the location of the sarcoma (upper extremity, lower extremity, body wall)?
5. Is the greatest dimension of the lesion equal or greater than 8 cm? (Y)
6. Is the Zubrod equal to or less than 1? (Y)
7. Have all required tests been performed within the time frame specified in Section 4.0? (Y)
8. Is there any evidence of metastatic disease (not including histologically undiagnosed lung lesions of ≤ 3 mm in diameter)? (N)
9. Are there any contraindications to surgery? (N)
10. Has the patient received any prior radiation, chemotherapy, or biotherapy? (N)
11. Has the patient had a previous malignancy other than adequately treated non-melanoma skin cancer or cervical cancer in-situ? (Y/N)
   - If yes, has the patient been disease free for ≥ 5 years? (Y)
12. Is the patient pregnant, lactating, or not using effective contraception? (code NA for men and for females without childbearing potential) (N/NA)
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 which would prevent informed consent or limit survival to less than 2 years? (N)
15. Has a study-specific consent been signed? (Y)
16. a) What is the WBC (per 1000)? (≥ 4)
   b) What is the ANC? (≥ 1800)
17. What is the platelet count (x 1000)? (≥ 150)
18. What is the bilirubin? (≤ 1.5)
19. What is the creatinine? (≤ 1.5)
20. What is the AST? (≤ 1.5 x ULN)
21. What is the ALT? (≤ 1.5 x ULN)
22. Does the patient have any known hypersensitivity to *e-coli* derived proteins?  

23. PT, PTT < 1.25 times normal (*prior to coumadin*)?  

24. Fibrin Split products < 2 x normal?  

25. Fibrinogen > 200 mg/dl?  

26. More than two weeks since minor surgery e.g. port placement; more than four weeks since major surgery?  

27. History of a bleeding or clotting diathesis?  

28. Cardiac ejection fraction > 50% *(If bypass surgery, was it done at least one year prior to enrollment and has the cardiologist determined stability of disease?)*  

The following questions will be asked at Study Registration:  

1. Name of institutional person registering this case?  

2. Has the Eligibility Checklist *(above)* been completed?  

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  

13. Zip Code  

14. Patient’s Insurance Status  

*(continued on next page)*  

RTOG S-0121  

**ELIGIBILITY CHECK** (8/1/01)
15. Will any component of the patient’s care be given at a military or VA facility?

16. Specify tumor location (lower extremity; upper extremity; body wall)

17. Will the patient be receiving IMRT?

18. Medical Oncologist

19. Treatment Start Date

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 8,100 newly diagnosed patients per year in the USA; this represents 0.66% of the invasive malignant neoplasms diagnosed per year. There are small numbers of a variety of histopathological types of soft tissue sarcomas at each anatomic site in the body. Approximately 60% of soft tissue sarcomas occur in the lower extremity and torso, with the remaining 40% being distributed throughout the remainder of the body. In recent years, rapidly evolving treatment strategies have generated considerable interest in the management of these relatively uncommon tumors.\(^1\) Historically, radical surgical resection has been the treatment of choice for soft tissue sarcomas. Enneking and Shiu from the University of Florida and Memorial Sloan Kettering Cancer Center have achieved some of the very best results using resection alone, with local recurrence rates of 17% and 18%, respectively. However, this was achieved with an amputation rate of 54% and 42%, respectively. Recent results of surgery alone include local control rates of 66% at Memorial Hospital and 69% at MGH.\(^5\) It is now generally accepted that limb salvage efforts should include surgery in combination with other modalities, especially pre-operative or post-operative radiation therapy.\(^7\) The rationale for combining radiation and surgery is that sarcomas, especially high grade sarcomas, infiltrate the grossly normal appearing tissue adjacent to the evident lesion. Thus, removal of the gross lesion by simple excision is followed by local recurrence in 70-90% of patients. Simple resection has been replaced by radical resection so that the surgical margins include a wide margin of grossly normal tissue around the tumor.\(^3,5\) Radiation at moderate dose levels (50-55 Gy) should be effective in eradicating the microscopic extensions beyond the gross lesion. In other words, moderate dose radiation and relatively conservative surgery should achieve the same result as the expansion from simple to radical surgery. This has been accomplished in a number of centers with low amputation rates and has been an effective method of limb salvage.\(^7,10\)

1.2 Combined Radiation Therapy and Surgery

Two hundred fifty-eight patients treated with wide resection and pre-operative or post-operative radiation therapy have been reported by Suit and his co-workers from the Massachusetts General Hospital (MGH) from 1971-1985.\(^11\) The five-year local control rates in post-operative and pre-operative groups were 86% and 91%, respectively. Moreover, the local success rate has been improved recently. Between 1980 and 1986, local control rates were 92% and 97% for patients treated postoperatively (63 patients) and preoperatively (82 patients), respectively. In this non-randomized trial, there appeared to be an advantage with the use of pre-operative radiation therapy, especially for larger lesions. This is also supported by work published by Barkley and co-workers at the M.D. Anderson Hospital.\(^12\) Their experience also indicates an advantage with regard to local control in patients receiving pre-operative radiation therapy compared to those receiving post-operative radiation therapy. In their series of 110 patients, the local recurrence rate was 10% in patients receiving pre-operative radiation therapy versus 20% in those patients receiving post-operative radiation therapy, and local control and disease-free survival rates were higher for patients with Stage IA, IIA or IIIA sarcomas. For Stage IIB and IIB tumors, local control was impressively higher for patients treated preoperatively. Disease-free survival was low for Stage IIB and IIIB patients; thus, 50% loss was due principally to distant metastases. Barkley has also found a correlation between survival and size and grade of sarcomas. For 13 patients with sarcomas less than 5 cm, the survival at 66 months was 90%. For patients with sarcomas of 5-15 cm, the survival was approximately 60%, but only 45% of patients with sarcomas >15 cm in diameter survived.

1.3 Combined Radiation Therapy, Chemotherapy, and Surgery

Chemotherapy has been used in an effort to improve both local and systemic control in patients with soft tissue sarcomas. Eilber and Morton have been strong proponents of a program which has consisted of intra-arterial adriamycin followed by rapid fraction radiation therapy and subsequent local excision.\(^13\) Their single institutional data has consistently shown local recurrence rates of ≤ 10% with survival rates of 74% in Stage III tumors. They have since shown that it is not necessary to provide the adriamycin by an intra-arterial route. Chemotherapy has been assessed extensively as a means of improving survival in patients with locally limited disease. Most studies assessing adjuvant chemotherapy in the management of soft tissue sarcomas have not demonstrated a significant improvement in survival.\(^14-18\) Many have demonstrated that chemotherapy given for the purpose of improving survival has improved disease-free survival and local control. In the metastatic setting, a combination of ifosfamide with mesna, doxorubicin, and dacarbazine has resulted in response rates as high as 47% with complete response rates as high as 10%.

In spite of this, progress has not been made toward the development of effective adjuvant chemotherapy strategies for soft tissue sarcoma. Metastasis occurs in up to half of all patients, even with adequate local control.\(^21,22\) Most hematogenous metastases are to the lung. The frequency with which distant failure occurs is directly proportional to the size of the primary tumor in patients with high-grade soft tissue sarcomas. An abstract
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 in which expression is restricted to endothelial cells, it is clear that the KDR tyrosine kinase receptor plays a critical role in angiogenesis. 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 describing a meta-analysis of 13 randomized trials of adjuvant chemotherapy versus control in soft tissue sarcomas demonstrated that chemotherapy increases overall survival by 9%, with both distant and local recurrence rates being reduced. There has been progress in the application of chemotherapy. Considerable interest has been focused on the ability to maintain dose intensity of chemotherapy using colony-stimulating factors to alleviate myelosuppression. Granulocyte-macrophage (GM-CSF) has been used with a variety of regimens to help maintain dose intensification. In a few studies, this has resulted in improved response rates.

The low overall incidence of soft tissue sarcomas and the fact that most patients do not participate in randomized clinical trials has made the overall efficacy of adjuvant chemotherapy for high-grade soft tissue sarcomas a difficult question to answer. Although a retrospective analysis would suggest little benefit, there are data supporting the concept for patients with high-risk extremity sarcomas. A large meta-analysis of adriamycin-based adjuvant chemotherapy reported a benefit for local recurrence-free survival, distant recurrence-free survival, and overall recurrence-free survival of 6%, 10%, and 10% respectively. A recent abstract report of a multicenter randomized trial of adjuvant chemotherapy vs. no chemotherapy in patients with high-risk soft tissue sarcomas indicated significant improvement in disease-free survival for the chemotherapy treated group. A recent abstract from a multi-institutional experience confirms an advantage for neoadjuvant chemotherapy and radiation therapy for local and distant disease control in these patients. A pilot study at Massachusetts General Hospital and a subsequent RTOG cooperative group multi-institutional phase II trial (RTOG 95-14) of combined neoadjuvant chemotherapy and radiation therapy for patients with > 8 cm grade 3 soft tissue sarcoma of the extremity and body wall have been completed. The pilot Massachusetts General Hospital study continues to mature in data collection, and a reported actuarial five-year disease-free survival rate of 84% compares quite well to their institutional historical control (non-chemotherapy treated patients) five-year disease-free survival of 45%. The RTOG study 95-14 was closed to patient accrual in February 2000 with an average accrual of 1.86 patients per month and with a total of 66 patients entered. Adult patients with Stage IIB or IIB extremity and body wall soft tissue sarcoma > 8 cm were treated with MAID chemotherapy for five days. Following a two day rest, radiation therapy to 22 Gy was delivered in 11 fractions. After two days, this chemotherapy/radiation cycle was repeated bringing the pre-operative radiation dose to 44 Gy and the number of chemotherapy cycles to three. After a three week rest, surgical resection was performed. If surgical margins were positive, an additional 16 Gy boost was delivered in 8 fractions followed by three additional chemotherapy cycles.

Short term interim analysis is as follows: 41 patients are evaluable (cases entered by April 1999) for preliminary data review at this time. 80% of the patients had high-risk large grade 3 sarcomas of soft tissue with a two year estimated local recurrence rate of 6% and a two year estimated overall survival (with death from any cause) of 95%. The most significant treatment-related toxicity was grade 4 neutropenia seen in approximately two-thirds of the patients during their chemotherapy cycles. Only two patients, however, did not complete neoadjuvant chemotherapy secondary to treatment-related problems. Importantly, 85% of the patients thus far had clear surgical margins and 93% had limb-sparing surgery despite the fact that the majority had high grade large extremity tumors. In a recent single institutional trial clear surgical margins and evidence of good histologic response within the primary tumor to neoadjuvant therapy in patients with IIB soft tissue sarcomas has translated into survival advantages. A recent abstract from a multi-institutional experience confirms an advantage for neoadjuvant chemotherapy and radiation therapy for local and distant disease control in these patients with high-risk soft tissue sarcomas. The RTOG study 95-14 with the addition of a compound that is not competitively toxic and may enhance this multimodality approach for these patients who historically have a five-year survival in the 30-50% range.
good bio-availability 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 a combined chemotherapy/radiation therapy treatment scheme. It has been suggested that these three modalities may be synergistic with additive clinical effect but without additive toxicity. These responses have been noted in both in vitro and in vivo studies. Synergistic effects have been documented with certain chemotherapy agents, notably alkalating agents and adriamycin. The predictability of the effectiveness of any anti-angiogenic agent on a particular soft tissue sarcoma according to size or grade is unknown at present. One can surmise that a soft tissue sarcoma displaying an angiogenic phenotype perhaps secondary to differential gene expression might prove to be sensitive to these anti-angiogenic agents. Although there is some early data to suggest that a high proportion of soft tissue sarcomas express an upregulation of angiogenesis by genomic analysis regardless of tumor grade, related clinical response data is lacking. 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 chemotherapy/radiation therapy, within the design of this study we propose to add the compound SU5416 to the treatment schema used for RTOG 95-14. This strategy takes into account the genetically fluid tumor cell compartment and the genetically stable (chemotherapy/radiation resistant) endothelial cell compartment. In addition, the low toxicity profile of SU5416 seen in phase I studies with lack of significant leukopenia and thrombocytopenia suggests non-competitive toxicity in this proposed trial making this compound an ideal combination drug for the MAID regimen used in RTOG 95-14 in which the major toxicity was neutropenia and thrombocytopenia. Since it is likely that any anti-angiogenic drug targeted to bulk tumor would be best suited clinically in a combined modality setting, this trial is designed to evaluate a cohort of patients with the same clinical eligibility established in RTOG 95-14. At the present time, there are several NCI sponsored trials evaluating the combination of SU5416 with chemotherapy for advanced disease, notably in colorectal cancer and previously treated measurable metastatic disease of any tumor type. This proposed trial will build on the previous database of RTOG 95-14 with the addition of SU5416 in a cooperative group setting. In addition to the clinical parameters of disease-free and overall survival, tissue and blood specimens will evaluate biological endpoints for the purpose of assessing the expression of angiogenic markers both pre- and post-treatment as a surrogate measure of response to SU5416. Tissue will be available from RTOG 95-14 to serve as a control group (chemotherapy/radiation therapy without SU5416) to detect any differences (see Appendix VII-XI). 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.

**2.0 OBJECTIVES**

2.1 To determine the maximum tolerated dose (MTD) of SU5416 with this chemotherapy/radiation therapy treatment scheme

2.2 To estimate the disease-free survival, local control, and overall survival rates in high-grade soft tissue sarcomas treated with MTD SU5416 added to the RTOG 95-14 protocol treatment. These results will be compared to a similarly treated cohort of patients from RTOG 95-14

2.3 To assess response to pre-operative radiotherapy/chemotherapy and SU5416 by using non-invasive imaging criteria pre- and post-delivery of combined modality therapy (utilizing the Response Evaluation Criteria in Solid Tumors [RECIST] criteria; see Section 11.2 and Appendix XII) as well as standard histologic criteria of response within the surgical specimen. This will be compared to a similarly treated cohort of patients from RTOG 95-14

2.4 To assess the toxicity of pre-operative combined chemotherapy and radiation and SU5416 using acute toxicity and late radiation morbidity scoring scheme. This will be compared to a similarly treated cohort of patients not receiving SU5416 (RTOG 95-14) and assessment of acute and chronic toxicity will include any assessment of surgical wound healing complications

2.5 To assess whether SU5416 has quantitative anti-angiogenic effects in vivo. This will be accomplished by systematic evaluation and comparison of biomarker tissue surrogates for microvascular changes prior to, during, and following combined modality therapy (chemotherapy, radiation therapy, SU5416). These will
include examination of paraffin embedded tumor and tumor stromal interface for angiogenic regulators (VEGF, KDR, FLT-1, and bFGF) and tumor endothelial cell proliferation (Ki67) as well TUNEL assay to assess apoptosis in both tumor and endothelial cells. The MVD (CD34) will be determined to assess peritumoral angiogenesis. In addition, ELISA will be used to quantitate circulating levels of angiogenic growth factors (VEGF, bFGF).

2.6 Archived paraffin-embedded surgical pathology specimens from RTOG 95-14 cohort (obtained pre- and post-combined modality therapy with identical chemotherapy and radiation therapy regimen) will be assessed for the same biomarker tissue surrogates (listed in section 2.5) in order to elucidate the contribution of SU5416 effect seen in this study.

3.0 PATIENT SELECTION

3.1 Eligibility Criteria

3.1.1 Patients must have a primary or recurrent (post surgery) soft tissue sarcoma confirmed by study pathologist as grade 2 or 3 on a grade 1-3 scale or grade 3-4 on a grade 3-4 scale (See Section 10.1.3). Open incisional biopsy is preferred; multiple core biopsies under CT guidance are acceptable, provided they are adequate for demonstration of tumor/stromal interface (See Section 8.1.1). Biopsy must be done within 2 months prior to registration (see Appendix VII-XI);

3.1.2 Sarcoma located on the upper (includes shoulder) or the lower (includes hip) extremities or on the body wall;

3.1.3 1998 AJCC Stage IIC and III (>8 cm) will be included in this study (see Appendix III);

3.1.4 On pre-operative chest CT scans, patients may have ≤ 4 chest lesions that are all ≤ 3 mm in diameter each and still be eligible for this protocol, providing there is no evidence of lung metastases. The lesions may be ≤1 cm if demonstrated to be stable over a period of one year and if they fit criteria for granulomas. This is allowed because of the propensity of most patients over 60 to have some parenchymal lesions that are not pathologic;

3.1.5 Zubrod performance status must be ≤ 1;

3.1.6 WBC ≥ 4,000 or ANC ≥ 1800, platelets ≥ 150,000, total bilirubin ≤ 1.5 mg/dL, serum creatinine ≤ 1.5 g/dL (if > 1.5 g/dL, then creatinine clearance should be > 60 ml/min), AST and ALT ≤ 1.5 x ULN, PT and PTT ≤1.25 times normal (prior to coumadin), Fibrin split products < 2 x normal/Fibrinogen >200 mg/dl;

3.1.7 Normal heart function (study of EF ≥ 50% within past six months). Patients with a history of atherosclerotic coronary artery disease requiring bypass surgery may only be enrolled provided the surgery occurred at least one year prior to enrollment and after consultation with a cardiologist to determine stability of disease;

3.1.8 Pretreatment evaluations must be completed as in Section 4.0, and treatment must begin within two weeks after registration;

3.1.9 Patient must sign a study-specific informed consent form prior to registration.

3.2 Exclusion Criteria

3.2.1 Prior treatment with radiation, chemotherapy, or biotherapy;

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 Histopathology of rhabdomyosarcoma, extraosseous Ewing’s, primitive neuroectodermal tumors, osteosarcoma or chondrosarcoma, Kaposi’s sarcoma or angiosarcoma of the scalp/face, any sarcoma of the head and neck;

3.2.4 Prior or concurrent malignancies (other than surgically treated carcinoma in situ of the cervix and squamous or basal cell carcinoma of the skin) within the preceding five years;

3.2.5 Serious medical or psychiatric illness which would prevent informed consent or limit survival to less than two years;

3.2.6 Uncompensated coronary artery disease on ECG or physical examination, history of myocardial infarction or severe/unstable angina in the past 6 months, congestive heart failure, LVEF ≤ 50%, or any cardiovascular abnormality resulting in a New York Heart Association Functional Status ≥ Class II (see Appendix II);

3.2.7 Active uncontrolled bacterial, viral, or fungal infection until these conditions are corrected or controlled;

3.2.8 Known hypersensitivity to E-coli derived proteins;

3.2.9 Contraindications to limb-salvage surgery;

3.2.10 History of a bleeding or clotting diathesis;

3.2.11 Patients must not be pregnant or breast feeding, and both genders must practice suitable contraception throughout the study. Women of childbearing potential must have a negative pregnancy test within 14 days of enrollment. The effect of SU5416 on the developing fetus and embryo are unknown. However
embryonic development is dependent on coordinated angiogenesis/neovascularization, and exposure to an anti-angiogenic agent may result in teratogenicity. The reported teratogenic effects associated with use of thalidomide may have resulted from interruption of neovascularization in the developing limb bud, via an anti-angiogenic effect of the drug. Excretion of 5115416 into breast milk has not been studied;

3.2.12 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 Plain films and i.v. enhanced MRI or computerized tomography (CT) of involved extremities prior to biopsy; CT is adequate for tumors of torso;

4.1.3 PA and lateral CXR;

4.1.4 CT scan of chest prior to registration in protocol;

4.1.5 CBC, differential and platelet count, PTT, PT (INR), AST, ALT, alkaline phosphatase, total bilirubin, serum creatinine, serum calcium, fibrin split products, fibrinogen;

4.1.6 EKG; pregnancy test as applicable;

4.1.7 Echocardiography or MUGA scan to evaluate LVEF;

4.1.8 Blood for analysis for circulating angiogenic factors VEGF and bFGF (see Appendix X);

4.1.9 Additional pre-operative 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 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 energy of photon beam. A wider area of bolus should be used if there is subcutaneous or cutaneous involvement.

6.1.3 Needle 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 to full dose, skin over areas commonly traumatized (e.g., the elbow, knee, shin, femoral neck).

6.2 Pre-operative Radiation Therapy

6.2.1 Treatment is to consist of two courses of external beam radiation therapy (EBRT) interdigitated between MAID courses 1 and 2 and between courses 2 and 3. Each course of EBRT will begin 3 days after completion of each cycles of MAID course (i.e., 2 days off, out of hospital without therapy) and consist of 22 Gy in 11 fractions (once a day) over 15 days. If treatment falls on a Saturday or Sunday, treatment can resume on Monday. The total pre-operative irradiation dose will be 44 Gy.

6.2.2 The target volume of radiation therapy will include the site of the primary lesion and those tissues suspected of involvement by microscopic disease to a clinically important probability. In addition to
physical exam findings, MRI scans or CT scans (less desirable) obtained during evaluation will be used in defining the target volume. The margins beyond clinically or radiologically-evident sarcoma will be 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 Post-operative external beam radiation therapy (EBRT) boost will be given for patients with positive 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. Chemotherapy can resume thereafter.

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 SU5416 should be administered at pre-operative tolerated dose with post-operative radiation therapy as well as with the post-operative chemotherapy.

6.4 Dose Specifications

6.4.1 For the 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.

7.0 DRUG THERAPY (MAID)/SU5416
RTOG institutional participation in chemotherapy studies must be in accordance with the Medical Oncology Quality Control guidelines stated in the RTOG Procedures Manual.

<table>
<thead>
<tr>
<th>TX</th>
<th>Cycle 1 Days</th>
<th>RT* Days</th>
<th>Cycle 2 Days</th>
<th>RT Days</th>
<th>Cycle 3 Days</th>
<th>Surg Day</th>
<th>Cycle 4 Days</th>
<th>Cycle 5 Days</th>
<th>Cycle 6 Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mesna</td>
<td>1-4</td>
<td>22-25</td>
<td>43-46</td>
<td>101-104</td>
<td>122-125</td>
<td>143-146</td>
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<tr>
<td>Dox</td>
<td>1-3</td>
<td>22-24</td>
<td>43-45</td>
<td>101-103</td>
<td>122-124</td>
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<td>43-45</td>
<td>101-103</td>
<td>122-124</td>
<td>143-145</td>
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<tr>
<td>SU5416</td>
<td>twice weekly</td>
<td>twice weekly</td>
<td>twice weekly stop 2 days prior to surgery</td>
<td>twice weekly</td>
<td>twice weekly</td>
<td>twice weekly until chemotherapy stops</td>
<td></td>
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</tbody>
</table>

7.1 Doses

7.1.1 Pre-operative Chemotherapy
Patients will receive a maximum of six cycles of MAID chemotherapy with concurrent SU5416. Three cycles will be administered preoperatively interdigitated with radiation therapy, and three cycles will be administered postoperatively. The post-operative chemotherapy (Section 7.1.1.9) should be instituted
between 21-35 days following surgery. Delays beyond 35 days will be considered a major protocol deviation.

7.1.1.1 **Mesna:** 2500 mg/m²/day as a continuous intravenous infusion administered for 4 days starting on day 1 of the drug cycle and repeated on day 22 (provided patients have recovered from their toxicities). Optional: the same dose of Mesna could be given over 12 hours on day 4 only. The daily Mesna dose equals the daily ifosfamide dose and is reduced in parallel if needed.

7.1.1.2 **Doxorubicin:** 20 mg/m²/day as a continuous intravenous infusion administered via a central line for 3 days starting on day 1 of the drug cycle and repeated on day 22 (provided patients have recovered from their toxicities).

7.1.1.3 **Ifosfamide:** 2500 mg/m²/day as a continuous intravenous infusion for 3 days starting on day 1 of the drug cycle and repeated on day 22 (provided patients have recovered from their toxicities).

7.1.1.4 **Suggested Hydration:** Four hours prior to the ifosfamide administration, begin hydration of with D5'NS, 2400 cc/day at 100 cc/hr until six hours following the completion of the continuous infusion of ifosfamide.

7.1.1.4 **DTIC:** 225 mg/m²/day as a continuous intravenous infusion administered via a central line for 3 days starting on day 1 of the drug cycle and repeated on day 22 (provided patients have recovered from their toxicities).

7.1.1.5 **G-CSF:** 5 mcg/kg/day administered as a subcutaneous injection starting on day 5 (24 hours after completion of the administration of the chemotherapy) and continuing daily until white blood cell count recovers (post nadir granulocyte or ANC count of >10,000) even if the GCSF is given concurrently with the radiation therapy. While the patients are receiving GCSF, white blood cell counts and differential counts should be checked at least twice weekly until the G-CSF is discontinued.

7.1.1.6 **SU5416:** Administered concurrently with the pre-operative chemotherapy and radiation therapy, as a single agent between end of cycle 3 (Day 46) until within 25 days of surgery (Day 75-78), and administered concurrently with post-operative chemotherapy and radiation therapy for patients with positive surgical margins (see Section 6.3). 85mg/m² in the first 10 patients (30% dose escalation to 110 mg/m² and then 30% dose escalation to 145mg/m² according to toxicity criteria and clinical design - see Section 7.1.1.7), as a 1 hour intravenous infusion (at a rate of 200 cc/hour until all of the dose is administered) via a central or peripheral line twice weekly on Mondays and Thursdays or Tuesdays and Fridays of each week throughout pre-operative chemotherapy and radiation therapy and as a single agent between end of cycle 3 (Day 46) until within 2-5 days of surgery (Days 75-78). 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 full speed. 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 1 hour after drug administration. No patients will be started on the 110 mg/m² dose level until there is evidence that SU55416 at the 85 mg/m² dose level has not had dose limiting toxicity (see Section 7.1.1.7). Similarly, no patients will be started on the 145 mg/m² dose until there is evidence that SU5416 at the 110 mg/m² dose level has not had a dose limiting toxicity (see Section 7.1.1.7).

Pre-treatment Drug Regimen

Pre-medications must be administered prior to SU5416 administration and include:
- 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 p.o 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 reinitiation of treatment should be 10 mg. In addition, low dose dexamethasone (0.5-1mg 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 daily as prophylaxis against thromboembolic phenomena (only during SU5416 administration; to be discontinued within 2 days of surgery).

7.1.7 Clinical Design

Built into the clinical design of this study is a phase I rapid dose escalation or dose reduction schema for SU5416. Prior phase I studies with SU5416 have indicated that the MTD is 145 mg/m² as a single agent. Data regarding dose levels in phase II studies in combination with chemotherapy is limited. However, there are trials of SU5416 in combination with paclitaxel with a starting dose at 65 mg/m² with a 35% dose escalation, and a phase II study of SU5416 in combination with 5FU and leucovorin starting at 85 mg/m² and now at 110 mg/m² with design to increase to 145 mg/m² if tolerated. Since clinical data for dose schedule is inconclusive and since grade 4 toxicity at 190 mg/m² for SU5416 alone is non-myelosuppressive and should not overlap with MAID, from a hematologic toxicity standpoint, it was determined with advice from the NCI to begin at a dose of 85 mg/m². The 190 mg/m² level of SU5416 results in dose limiting toxicity of headache, nausea, and vomiting which was relieved at a 30% dose reduction. The design of the present study will begin at a dose level of 85 mg/m² during cycle 1 presurgery, which represents 60% of the known MTD dose. Doses of SU5416 should not be escalated within individual patients once treatment has been initiated. Toxicities arising during any presurgery cycle that include the administration of SU5416, chemotherapy, and radiation therapy will be used to determine the MTD of the combination of these treatments. Dose limiting toxicity (DLT) for the purpose of this study will be defined as grade 3 or greater nonhematologic (including hepatic) toxicity and grade 4 neutropenia and thrombocytopenia requiring hospitalization for sepsis syndrome or organ infection that are thought to be due to SU5416. Drug should be held until resolution of the DLT to baseline or grade 1, at which time drug is restarted at the next lowest dose level (i.e. 30% reduction in dose: 145 / 110 / 85 / 60 mg/m²; see Section 7.8 for a list of specific toxicities). Subsequent cycles will then continue with reduced dose of SU5416 (or other chemotherapy agents as noted in Section 7.8 based on type of toxicity) as tolerated. Any lethal toxicity associated with drug will put the study on hold until formal evaluation takes place. Therefore, the first 6 patients will be assigned to the initial dose level of 85 mg/m², and toxicity will be evaluated in cohorts of 3 patients. If there is no observed DLT in the first 3 patients, then the dose will be escalated by 30% to 110 mg/m². If, however, one DLT is seen in the first 3 patients at 85 mg/m², then 3 more patients will be evaluated at 85 mg/m² level, and if in the next 3 patients there is no further DLT noted, the dose will be escalated by 30% to 110 mg/m² (see Section 13.3.2). If in the second group of 3 patients at 85 mg/m², one patient experiences DLT, then there will be a dose reduction by 30% to 60 mg/m² which will be considered the MTD for the study. In addition, a dose reduction by 30% to 60 mg/m² is indicated if 2 or 3 of the first 3 patients experience DLT. This design will be re-tested at each 30% dose increase to maximum of 145 mg/m². If neither the 2 dose levels (110 mg/m² or 145 mg/m²) yields greater than one DLT in the total of 6 patients, then the dose will be reduced by 30% and that reduced level will be the MTD for this study. The design will accept no greater than one DLT for the MTD level determination (see Section 13.3.2). After the MTD for this study (within approximately the first 6-18 patients) has been established, then any DLT on an individual patient basis will require a dose reduction by 30% (see Section 7.7). The dose determination of SU5416 in the combined modality group will be assigned by Headquarters of RTOG when patient is registered for the study. At the end of the Phase I portion of the study, an amendment will be submitted that summarizes the results of the Phase I portion of the protocol and that delineates the rationale for the dose of SU5416 to be used for the Phase II portion of the protocol.

7.1.8 Drug Mixing

Both mesna and ifosfamide can be mixed together in one liter D₅W and administered via peripheral line. Both doxorubicin and DTIC can be mixed together in 1 liter NS and administered via a central line (should be protected from light).

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.

The actual administration set used will depend upon type 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.

7.1.9 Post-operative Chemotherapy
Patients who have had stable disease, or a minor or major clinical response to chemotherapy (including radiation therapy) will receive three cycles of treatment in the post-operative period. Post-operative chemotherapy should begin between 21-35 days after surgery. Each cycle of chemotherapy will include mesna, doxorubicin, ifosfamide, DTIC and G-CSF exactly as given in the pre-operative period. SU5416 will also be given in exactly the same dose and schedule as preoperatively during the adjuvant chemotherapy.

7.2 Mesna (Mesna)

7.2.1 Dose Formulation: Mesna is available as an injectable sterile preservative-free aqueous solution. The colorless solution is supplied in clear glass ampoules containing 4 and 10 ml of a 100 mg/ml solution. Mesna may be further diluted in 5% dextrose, 5% dextrose and 0.45% normal saline or normal saline to a final concentration of 1 to 20 ml. Mesna should be given as a continuous intravenous infusion via a peripheral line.

7.2.2 Mechanism of Action: Mesna is a uroprotective agent used to prevent hemorrhagic cystitis induced by the oxasphosphorines (Ifosfamide, cyclophosphamide). It has no intrinsic cytotoxicity, no antagonistic effects on radiotherapy or chemotherapy. Mesna binds with acrolein, the urotoxic metabolite produced by the oxasphosphorines to produce a non-toxic thioether, and slows the rate of acrolein formation by combining with 4-hydroxy metabolites of oxasphosphorines.

7.2.3 Drug Availability: Mesna is commercially available in 4 and 10 ml ampoules containing 100 mg/ml.

7.2.4 Storage: Intact ampoules are stored at room temperature. Diluted solutions are physically and chemically stable for 24 hours under refrigeration.

7.2.5 Side Effects: At the doses used for uroprotection, mesna is virtually non-toxic. However, adverse effects that have been attributable to mesna include: nausea, vomiting, diarrhea, abdominal pain, rash, lethargy, headache, arthralgia, myalgias, fatigue, and bad taste in mouth.

7.3 Doxorubicin (Adriamycin, Rubex)

7.3.1 Dose Formulation: Doxorubicin is available as a red powder for injection in 10, 20, 50, 100, and 150 mg vials. Five, 10, 25, 50, or 75 ml of preservative-free normal saline to the 10, 20, 50, 100, or 150 mg vials respectively to produce a solution containing 2 mg/ml. Doxorubicin should be given as a continuous intravenous infusion via a central line.
7.3.2 **Mechanisms of Action:** Doxorubicin is an anthracycline antibiotic. It causes intercalation between adjoining nucleotide pairs in the DNA helix causing inhibition of DNA and DNA-dependent RNA synthesis. Free radical generation is responsible for cardiac toxicity. Doxorubicin also inhibits topoisomerase II.

7.3.3 **Drug Availability:** Doxorubicin is commercially available.

7.3.4 **Storage:** Adriamycin RDF or Rubex intact vials are stable if protected from light at room temperature. Reconstituted solutions are stable for 24 hours at room temperature and 48 hours under refrigeration. The Adriamycin RDF 150 mg multidose vial is stable after reconstitution for 7 days at room temperature or 15 days if refrigerated and protected from sunlight.

7.3.5 **Side Effects:**
1. **Hematologic:** Leukopenia *(dose-limiting)*, also thrombocytopenia and anemia. Nadir 10-14 days, recovery in 21 days.
2. **Dermatologic:** Alopecia, usually complete; hyperpigmentation of nailbeds and dermal creases; radiation recall.
3. **Gastrointestinal:** Nausea and vomiting, sometimes severe; anorexia; diarrhea; mucositis, especially with daily x 3 schedule.
4. **Cardiovascular:** Arrhythmias, ECG changes; rarely, sudden death. Congestive heart failure due to mediastinal irradiation pre-existing cardiac disease, advanced age; risk is reduced with weekly or continuous infusion regimens.
5. **Other:** Red discoloration of urine; fever; anaphylactoid reaction; may enhance cyclophosphamide cystitis or mercaptopurine hepatotoxicity.
6. **Local effects:** Vesicant if extravasated; flush along vein, facial flush.

7.4 **Ifosfamide (Ifex)**

7.4.1 **Dose Formation:** Ifosfamide is available as a white crystalline powder in 1 and 3 gram single dose vials. When the 1 and 3 gram vials are reconstituted with 20 and 60 ml of sterile water respectively, each vial will contain 50 mg/ml. The solution's pH is approximately 6. Ifosfamide should be given as a continuous intravenous infusion via a peripheral line.

7.4.2 **Mechanism of Action:** Ifosfamide is an alkylating agent which is activated by hepatic microsomal enzymes to reactive alkylating substance. The reactive metabolites, ifosfamide mustard and aldophosphamide, are capable of covalent binding and cross-linking of DNA and cellular proteins.

7.4.3 **Drug Availability:** Ifosfamide is commercially available.

7.4.4 **Storage:** The intact, unreconstituted vials are stored at room temperature. The sterile reconstituted solution is stable for 1 week at 30-C or 3 weeks at 5-C. Ifosfamide liquefies at temperatures above 35-C.

7.4.5 **Side Effects:**
1. **Hematologic:** Leukopenia, thrombocytopenia *(dose-limiting)*; anemia.
2. **Dermatologic:** Alopecia, rash, urticaria.
3. **Gastrointestinal:** Nausea, vomiting, anorexia, constipation, diarrhea, salivation, stomatitis.
4. **Hepatic:** Elevated SGOT and SGPT, hyperbilirubinemia.
5. **Genitourinary:** Hemorrhagic cystitis *(incidence related to dose and schedule; more common with a single high dose)*; elevated creatinine.
6. **Neurologic:** Somnolence, lethargy, disorientation, confusion, dizziness, malaise.
7. **Other:** Hyponatremia, hypokalemia, phlebitis, fever, hypo- or hypertension.

7.5 **Dacarbazine (DTIC)**

7.5.1 **Dose Formulation:** The drug is available in vials containing 100 mg, 200 mg, or 500 mg of Lyophilized drug. The 100, 200, and 500 mg vials are diluted with 9.9, 19.7, and 49.5 ml of sterile water respectively, resulting in a concentration of 10 mg/ml. Protect the drug from direct light. Do not freeze. Discard if the solution turns pink/red. DTIC should be given as a continuous intravenous infusion via a central line.

7.5.2 **Mechanism of Action:** DTIC is classified as an alkylating agent. Activity may be the result of a least 3 mechanisms: *(1)* alkylation; *(2)* antimetabolite activity as a purine precursor; and *(3)* interaction with sulphydryl *(SH)* groups in proteins. Dacarbazine appears to be more active in G2 phase but is not particularly cell cycle phase specific.

7.5.3 **Drug Availability:** DTIC is commercially available. If there is any difficulty in obtaining DTIC for protocol use, please contact June Brouillette at Bayer Corporation *(203/812-2355)*.

7.5.4 **Storage:** Store vials under refrigeration and protected from light. In solution, dacarbazine is stable for 96 hours if refrigerated and protected from light, 24 hours if not refrigerated but protected from light. When further diluted in 500 ml *D*W or *NS*, it is stable for 24 hours if refrigerated, and 8 hours at room temperature and protected from light.
**Photodegradation:** The manufacturer of dacarbazine states that the drug does not decompose when left at room temperature under normal lighting conditions for eight hours.

**Note:** A change in color of solution from pale yellow to pink is indicative of decomposition of the drug.

### 7.5.5 Incompatibility
Metabolism of dacarbazine may be inducted by phenytoin or phenobarbital. Toxicity may be enhanced if given concomitantly with allopurinol, azathioprine, or mercaptopurine. Dacarbazine is physically incompatible with hydrocortisone sodium succinate and heparin.

### 7.5.6 Side Effects
1. Hematologic: Myelosuppression; nadir of WBC and platelet depression occurs approximately 21-25 days of treatment.
2. Dermatologic: Alopecia; facial flushing; extravasation may result in severe pain but has not resulted in tissue damage. Rapid i.v. push may result in pain along injection site or thrombophlebitis.
3. Gastrointestinal: Severe nausea and vomiting which characteristically lessens with each subsequent daily dose.
4. Hepatic: Increased SGOT, SGPT.
5. Renal: Increased serum creatinine, BUN.
7. Other: Flu-like syndrome (*with fever, malaise, myalgia*) rarely occurs approximately about 7 days after treatment and lasts 1-3 weeks. Rarely, anaphylaxis.

### 7.6 G-CSF - Filgrastim (*r-metHuG-CSF, Neupogen*)

#### 7.6.1 Dose Formulation
Commercial Neupogen® is available in 1 ml and 1.6 ml vials at a concentration of 300 mcg/ml. Discard unused portions. Use only one dose per vial; do not re-enter the vial. Do not save unused drug for later administration.

Neupogen® is also available as single-dose, preservative-free, pre-filled syringes with 26 gauge, 5/8 inch needles containing 300 mcg (0.5 ml) of Filgrastim (600 mcg/ml) and 480 mcg (0.8 ml) of Filgrastim (600 mcg/ml). If required, Neupogen® may be diluted in 5% dextrose. Neupogen® diluted to concentrations between 5 and 15 mcg/ml should be protected from adsorption to plastic materials by addition of albumin (human) to a final concentration of 2 mg/ml. **Do not dilute with saline at any time; product may precipitate.** For this study, G-CSF will be supplied in 480 mcg/1.6 ml vials; initial order quantities will be 100 vials; reorder quantities will be in 30 vial increments.

#### 7.6.2 Mechanism of Action
Filgrastim is a human granulocyte colony stimulating factor (*G-CSF*), produced by recombinant DNA technology. Neupogen® is the Amgen Inc. trademark for Filgrastim, recombinant metionyl human granulocyte colony stimulating factor (*r-metHuG-CSF*).

#### 7.6.3 Drug Availability
(*9/12/01*) G-CSF (*Filgrastim*) is commercially available. However, for this study it is being supplied free of charge by Amgen, Inc. and is available from UintaVision. To obtain a supply of G-CSF, complete the G-CSF (*Filgrastim*) Drug Request Form supplied in Appendix XIV, and fax or send the form to the following address:

UintaVision, Inc./Axion, Inc.  
232 Castro Street, Suite #2  
San Francisco, CA 94114  
General Phone: (800) 370-2508  
Fax: (650) 745-3877

UintaVision’s office hours are 6:30 a.m. to 1 p.m. PST; a phone message may be left at other times. Phone messages after 1 p.m. will be returned the next business day.

Orders received by 11:30 a.m. PST Monday through Thursday will be shipped for next day delivery. Orders received by 11:30 a.m. PST on Friday will be shipped for receipt the following Monday. **G-CSF orders from USA sites only will be accepted.** Patients must be registered to the study before study drug can be obtained. When the study is terminated, unused drug at the site will be returned to UintaVision, Inc./Axion, Inc. with a completed Return Medication Packing Slip (*see Appendix XV*) included to identify for which study the drug was originally shipped.

#### 7.6.4 Storage
Neupogen® should be stored in the refrigerator at 2-8 degrees Centigrade (36-46 degrees Fahrenheit). **Do not freeze. Avoid shaking.** Prior to injection, Neupogen® may be allowed to reach room temperature for a maximum of 24 hours. Any vial left at room temperature for greater than 24 hours should be discarded.

#### 7.6.5 Side Effects:
Neupogen® is contraindicated in patients with known hypersensitivity to E. coli-derived products, Filgrastim, or any component of the product. The only consistently observed clinical toxicity described with Neupogen® is medullary bone pain. Other clinical toxicities that have been described include skin rash, and cutaneous vasculitis. Since commercial introduction of Neupogen®, there have been rare reports of allergic-type reactions. Biochemical abnormalities that may occur include increases in alkaline phosphatase, uric acid, and lactate dehydrogenase.

7.7 SU5416 (NSC #696819; IND#59025)

7.7.1 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; polyoxy 35 castor oil (Cremophor-EL®); benzyl alcohol and dehydrated alcohol. Each vial is intended for single use only. The molecular formula of SU5416 is C15H14N2O. The formula weight is 238.29. SU5416 is a yellow-orange sterile parenteral formulation.

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 some of the sets tested, which are labeled as Nitroglycerine Sets, paclitaxel infusions were run through the study sets and the effluent analyzed by HPLC for leached DEHP plasticizer. The following sets had significant and unacceptable amounts of leached DEHP: Baxter vented nitroglycerin (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 Nitroglycerin 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 regarding specific manufacturer products compatible with paclitaxel infusions.

7.7.2 Preparation: It is very important that the drug be added to an empty bag first and the diluent added afterwards to avoid any chances of precipitation. If the diluent is added first followed by the drug, the mixture will precipitate. Using routine aseptic procedures, aspirate the correct volume of SU5416 into the syringe. Inject solution into an empty non-PVC plastic intravenous bag. As empty glass bottles contain residual amounts of saline or water, they are not compatible with SU5416 drug product. 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 into a peripheral vein). Invert and right the bag until the resulting solution is well mixed (15-20 times). Sugen 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 a 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 of 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.

7.7.3 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 endothelial cell. Binding of vascular endothelial growth factor (VEGF) to the extracellular 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 arrange from 35-235 minutes.

### Drug 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 DeBellas, R.Ph., Clinical Research Pharmacist, CTEP, at (301) 496-5725.

### Storage

Vials of SU5416 must be stored at controlled room temperature (22-28°C), protected from light.

### Stability

Diluted drug product is stable for 8 hours at room temperature.

### Side Effects

The dose limiting toxicity for SU5416 is nausea, headache, and vomiting.

#### Neurologic

Headache (can be 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, cerebral bleeding have been reported although these occurred in patients with underlying cancer + cardiovascular disease, so it is not clear that these are drug induced.

#### Respiratory

Dyspnea, cough, pharyngitis, voice alterations, pneumonia, hemoptyisis. 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 transminases, alkaline phosphatase, total bilirubin, a recent report of hepatic rupture possible secondary to SU5416 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

#### Musculoskeletal

Injection site pain

Toxicities due to excipients in the formulation such as Cremophor® or absolute alcohol were 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. Anaphylactic and severe hypersensitivity reactions have been reported in 2% of patients receiving paclitaxel in clinical trials. Patients receiving SU5416 are at risk for anaphylaxis and hypersensitivity reactions. Two patients to date reported with SU5416 have experienced an anaphylactoid reaction with SU5416, which were reversed with diphenhydramine and dexamethasone. Other hypersensitivity reactions possibly attributable to SU5416 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 held for observation 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, arthralgias, dizziness/vertigo, peripheral edema (all 17%), increased sweating (11%), myalgias, hypokalemia, pruritus, emotional lability, leukocytosis, abnormal vision, and amblyopia (all less than 10%).

No specific toxicities arising from antiangiogenic effects were noted. No effects on menstruation or fertility were observed. Patients with uncompensated coronary artery disease, 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.
7.8 Drug Dose Modification

The doses of chemotherapy will be attenuated as noted below. The most severe toxicity should determine the degree of attenuation.

7.8.1 Hematology Toxicity

7.8.1.1 Ifosfamide (and Mesna in parallel), Doxorubicin and DTIC doses are to be modified based both on the nadir (day 14) counts of the previous cycle and counts obtained on the day treatment is given. No new treatment course may begin unless the patient's granulocyte count is > 1500/ml and platelet count is >100,000/ml. If these are not present on day 22, then repeat counts weekly; if after 2 weeks the patient's counts are not adequate for therapy, contact Dr. Ettinger.

### Absolute Neutrophil Count
(Reduce doses only if ANC is <1000 for >5 days)

<table>
<thead>
<tr>
<th>ANC Nadir of Last Course</th>
<th>ANC Day 1 of Each Cycle</th>
<th>% Dose to Give</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ 1500</td>
<td>&gt; 1500</td>
<td></td>
</tr>
<tr>
<td>&gt; 1000</td>
<td>0 %</td>
<td>100%</td>
</tr>
<tr>
<td>500-1000</td>
<td>0 %</td>
<td>80%</td>
</tr>
<tr>
<td>&lt; 500</td>
<td>0 %</td>
<td>70%</td>
</tr>
</tbody>
</table>

If the patient has an ANC < 1000, the CBC should be repeated 3 times weekly until the ANC is > 1500. There will be a dose reduction only if the ANC remains below 1000 for greater than 5 days.

### Platelets

<table>
<thead>
<tr>
<th>Platelets Nadir of Last Course</th>
<th>Platelets Day 1 of Each Cycle</th>
<th>% Dose to Give</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ 100,000</td>
<td>&gt; 100,000</td>
<td></td>
</tr>
<tr>
<td>&gt; 75,000</td>
<td>0 %</td>
<td>100%</td>
</tr>
<tr>
<td>50,000-75,000</td>
<td>0 %</td>
<td>80%</td>
</tr>
<tr>
<td>&lt; 50,000</td>
<td>0 %</td>
<td>70%</td>
</tr>
</tbody>
</table>

In the event that dose adjustments are needed for both ANC and platelets, patients are to receive the lower dose. Treatment should be delayed for one week until ANC is >1500, and the platelet count is >100,000. If after one week the counts have recovered, the patient should proceed with the next course of treatment (Day 1) based on the previous course's nadir counts. However, if the counts have not recovered in two weeks, the Study Coordinator should be contacted. Patients and investigators need to be attentive to the possibility of fever and infection so that these complications can be promptly and appropriately managed.

When a dose reduction is made for a decreased ANC or platelet count and the reduced dosage results in no toxicity, the next course should be given at intermediate-dose rather than full-dose, e.g., if a 30% dose reduction results in no toxicity, the next course should start at 80% dose rather than 100% (i.e. 70% dose increased to 80% of dose, and 80% dose would be increased to 100%).

Dose reductions are not based on a single nadir count. The ANC must remain < 1,000 for > 5 days before a dose reduction is made.

If chemotherapy must be withheld due to hematologic toxicity, CBC and platelet counts should be obtained weekly until the counts reach the lower limits for treatment as outlined. The treatment schedule will then proceed in the usual sequence.

7.8.2 Gastrointestinal Toxicity (Ifosfamide, Doxorubicin, DTIC, SU5416)

Nausea and/or vomiting should be controlled with adequate antiemetics. If grade 3 nausea/vomiting occurs in spite of antiemetics, the dose should be reduced by 30% for the next course. If tolerated, increase back to 100% dose as soon as possible. If the toxicity recurs, reduce the dose by 30%. If toxicity returns at this dose, discontinue drug.

If on Day 1 of any treatment cycle the patient has mucositis, the treatment should be withheld until the mucositis is cleared. If acute grade 3 mucositis occurs at any time, the dose should be given at 75% dose until the mucositis is completely cleared. Drug can be give at full dose for subsequent cycles.

7.8.3 Hepatic Toxicity

Give the following percent of previous course's dose based on the patient's bilirubin the day of treatment.

Dose limiting toxicity (DLT) for the purposes of this study will be defined as Bilirubin > 3.0 - < 5.0 mg/dl
that is thought to be due to the SU5416. Drug is held until resolution of the DLT to baseline, at which time drug is restarted at the next lowest dose level (i.e. 30% reduction in dose: 145 → 110 → 85 → 60 mg/m^2).

<table>
<thead>
<tr>
<th>Liver Function Test Result</th>
<th>% Agent to Give</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bilirubin (mg/dl)</td>
<td>SU5416</td>
</tr>
<tr>
<td>&gt;1.5 - &lt;3.0</td>
<td>100%</td>
</tr>
<tr>
<td>&gt;3.0 - &lt;5.0</td>
<td>70%</td>
</tr>
<tr>
<td>5.0</td>
<td>0%</td>
</tr>
</tbody>
</table>

### Neurotoxicity

Ifosfamide doses will be modified for neurotoxicity as outlined below:

#### 7.8.4.1 Mild Somnolence (sleeping constantly but easily aroused and oriented)
Decrease dose of narcotics or antiemetics; continue ifosfamide with no change in dose.

#### 7.8.4.2 Moderate Somnolence (difficult to arouse or disoriented when finally awakened)
Discontinue IFOS until toxicity clears and then reinstitute at same dose. If moderate somnolence recurs, again discontinue IFOS and reinstitute at a 25% dose reduction for the rest of the course. If the same symptoms recur at the reduced dose, again hold until symptoms resolve and restart at a 25% further dose reduction. If symptoms recur after a 50% reduction, discontinue permanently.

#### 7.8.4.3 Visual Hallucinations, Confusion, Catatonia
Hold IFOS, reinstitute at 25% reduced dose with next course, minimizing any other psychoactive medications. If symptoms recur, decrease IFOS dose by another 25% with the subsequent course. If symptoms recur after a 50% dose reduction, discontinue permanently.

#### 7.8.4.4 Headaches (SU5416)
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 treated 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.

### Cardiovascular Toxicity

#### Cardiac Events
Doxorubicin should be withheld if EKG abnormalities or congestive heart failure develops. Non-invasive ventricular function studies should be performed if available. If EKG abnormalities improve, Doxorubicin may be reinstituted, but it should not be reinstituted if congestive heart failure or ventricular dysfunction are present. Patients whose MUGA scan drops to less than 50% should be removed from the study.

#### Thromboembolic events
Treatment and prophylaxis using appropriate anticoagulation should be initiated, but SU5416 should be discontinued only when thrombotic events, 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 when any thromboembolic events occur.

### Genitourinary

Cystitis: IFOS-related gross or microscopic hematuria correlates with the concentration of drug metabolites in the bladder. Adequately hydrate patients, and ensure frequent voiding. Should grade 2 hemorrhagic cystitis occur, discontinue IFOS. Reinstitute IFOS at a 50% reduced dose when hematuria has cleared. If grade 2-3 bladder toxicity occurs at the 50% dose reduction, discontinue IFOS permanently. IFOS may be escalated to full dose after dose reduction with subsequent courses if no hematuria occurs at the 50% dose reduction.

#### 7.8.6.1 Nephrotoxicity
Give the following percent of the previous course’s dose for nephrotoxicity based on renal function on the day of treatment:

<table>
<thead>
<tr>
<th>Serum Creatinine (mg/dl)</th>
<th>Doxorubicin</th>
<th>DTIC</th>
<th>Ifosfamide</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤1.5</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>1.5-2.0</td>
<td>100%</td>
<td>100%</td>
<td>75%</td>
</tr>
<tr>
<td>2.1-3.0</td>
<td>100%</td>
<td>100%</td>
<td>50%</td>
</tr>
<tr>
<td>&gt;3.0</td>
<td>100%</td>
<td>100%</td>
<td>0%</td>
</tr>
</tbody>
</table>
7.8.7 Miscellaneous Toxicity

7.8.7.1 Skin Ulceration/Phlebitis: Doxorubicin may cause chemical phlebitis, even when administered by continuous infusion through side-arm of a rapidly running intravenous infusion. Chemical phlebitis is not an indication to stop any drug. Doxorubicin must be administered through central line (not merely a long line).

7.8.7.2 Extravasation outside a vein will cause skin necrosis; stop the infusion immediately if extravasation is suspected.

7.8.7.3 Fever and Flu-like Syndrome associated with DTIC administration may be avoided with oral acetaminophen.

7.8.7.4 Alopecia: IFOS and Doxorubicin cause total alopecia.

7.8.7.5 For any grade 3 or 4 toxicity not mentioned above, the treatment should be withheld until the patient recovers completely or to grade 1 toxicity. The treatment should then be resumed at 50% dose (permanent dose reduction). For grade 1 or 2 toxicities, no dose reduction should be made.

7.8.7.6 Hypersensitivity reactions: All patients experiencing ≥ grade 3 hypersensitivity reactions thought to be due to SU5416 will be reported to CTEP as an adverse event. All patients experiencing grade 4 hypersensitivity reaction will be removed from study. If a patient experiences a grade 3 hypersensitivity reaction at a reduced dexamethasone dose, 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 study and reported to RTOG headquarters and IDB.

7.8.7.7 Respiratory: The main cause of shortness of breath may be related to hypersensitivity reactions (see Section 7.8.7.6). To date, SU5416 has not been associated with specific pulmonary toxicity.

7.8.7.8 Metabolic: Reduce SU5416 by 30% for hypercalcemia between 12.5 and 13.

7.9 Disease Progression

Patients who show progressive disease at the primary site during the pre-operative period will not receive post-operative chemotherapy. If progressive disease is noted after any pre-operative cycle, the patient will be offered surgical therapy immediately. Patients who develop systemic metastatic disease will be considered treatment failures and will be removed from protocol treatment, but followup data will still be collected. They may be treated with other forms of palliative chemotherapy.

7.10 Adverse Drug Reaction Reporting

7.10.1 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 IV for protocol reporting guidelines.

7.10.2 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.

7.10.3 Reporting

7.10.3.1 Expedited Reporting for Phase 1 Studies (Including hospitalization defined in bullet 1 below):

<table>
<thead>
<tr>
<th><strong>EXPECTED EVENTS</strong></th>
<th><strong>Grades 2 – 3</strong></th>
<th><strong>Grades 4 and 5</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Attributon Possible, Probable, or Definite</td>
<td>Regardless of Attribution</td>
<td>Grade 1 - 3</td>
</tr>
<tr>
<td>Grayscale 2 - Expedited report within 10 working days.</td>
<td>Report by phone to IDB and RTOG within 24 hours. Expedited report to follow within 10 working days.</td>
<td>Adverse Event Expedited Reporting NOT required.</td>
</tr>
<tr>
<td>Grayscale 3 - Report by phone to IDB within 24 hours. Expedited report to follow within 10 working days.</td>
<td>This includes deaths within 30 days of the last dose of treatment with an investigational agent.</td>
<td>Report by phone to IDB and RTOG within 24 hours. Expedited report to follow within 10 working days.</td>
</tr>
</tbody>
</table>

**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: Investigational Drug Branch, Post Office Box 30012, Bethesda, Maryland, 20824, and to RTOG headquarters.

### Note 4
A list of agent specific expected adverse events can be found in the protocol document or consent form.

### Note 5
Reactions judged definitely 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](http://ctep.info.nih.gov). The NCI Guidelines for expedited adverse reporting are also available at this site.

#### 7.10.3.2 Expedited Reporting for Phase II Studies (Including hospitalization defined in bullet 1 below):

<table>
<thead>
<tr>
<th>Unexpected Events</th>
<th>Expected Events</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Grades 2 – 3</strong>&lt;br&gt;Attribution Possible, Probable, or Definite</td>
<td><strong>Grades 1 - 3</strong>&lt;br&gt;Regardless of Attribution</td>
</tr>
<tr>
<td>Expedited report within 10 working days.</td>
<td>Adverse Event Expedited Reporting NOT required.</td>
</tr>
<tr>
<td><strong>(Grade 1 - Adverse Event Expedited Reporting NOT required.)</strong></td>
<td>Expedited report to IDB and RTOG, including <strong>Grade 5</strong> Aplasia in leukemia patients, within 10 working days.</td>
</tr>
<tr>
<td>Report by phone to IDB and RTOG within 24 hours. Expedited report to follow within 10 working days.</td>
<td><strong>Grade 4</strong> Myelosuppression is not to be reported, but should be submitted as part of the study results.</td>
</tr>
</tbody>
</table>

- Telephone number available 24 hours daily: **(301) 230-2330** (*Recorder after hours*).  
- See the DCTD/NCI Common Toxicity Criteria  
- Report to: Investigational Drug Branch, Post Office Box 30012, Bethesda, Maryland, 20824, and to RTOG headquarters.

- A list of agent specific expected adverse events can be found in the protocol document or consent form.

- Reactions judged definitely not treatment-related should not be reported. However, a report should be submitted if there is reasonable suspicion of drug effect.

- Expedited reports are to be submitted using AdEERS or the paper templates available at [http://ctep.info.nih.gov](http://ctep.info.nih.gov). The NCI Guidelines for expedited adverse reporting are also available at this site.

#### 7.11 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”):

a) 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 NCT’s participation in the proposed combination protocol.

b) 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.

c) 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.

d) 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 Biopsy

8.1.1 Biopsy ideally should be an open incisional biopsy. Multiple core biopsies under CT guidance are acceptable, provided they are adequate for demonstration of 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 open biopsy is preferred 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 Resection of the sarcoma will occur following combined pre-operative chemotherapy, Sugen 5416, and radiation (Day 80; surgery may be delayed for up to two additional weeks, i.e., Day 94, but patient should continue to receive SU5416 to within 2-5 days of any surgery date). 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.
8.2.2 The surgeon and radiation oncologist will consult after diagnosis and prior to the institution of pre-operative therapy. Following pre-operative 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.

8.2.3 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 Amputation
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 post-operative radiation and chemotherapy 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 pre-operative 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 post-operative 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 (Davol, Hemavac, 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 post-operative management of these patients:
- Maintain staples or skin sutures per surgeon preference but because of potential delay in wound healing, 3-4 weeks is recommended.
- 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 limb salvage procedure. For other anatomic areas, it must be the 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 80 after start of pre-operative treatment

8.5.2 *Minor Variation* - Surgery completed > day 80-day 94 after start of pre-operative treatment

8.5.3 *Major Deviation* - Surgery completed > day 94 after pre-operative treatment start.

9.0 OTHER THERAPY

Not applicable to this study.

10.0 PATHOLOGY

10.1 Assessment of Pre-treatment Biopsy Specimen

10.1.1 Central Pathology Review (Upon Study Completion)

a) Histology slides and a copy of the surgical pathology report must be mailed with the pathology submission form 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 Histopathologic Assessment

a) Sarcoma phenotype as categorized by the WHO *(1994)*

b) Histologic grade *(grade 2 or 3)* on a scale of 1-3 or grades 3-4 on a scale of 1-4. 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 at least intermediate grade *(2 or 3)* on a scale of 1-3. Grades 2 and 3 tumors may be separated by features such as mitotic rate *(usually > 6 mitotic figures per 10 HPF)*, percent necrosis, cellularity, pleomorphism, and differentiation. Mitotic rate and necrosis appear to be the most important prognostic factors, and should be useful for separating grade 2 from 3. Separating grades 2 and 3 based upon presence or absence of necrosis is recommended. Another 3 scale system employs degree of tumor differentiation, mitotic activity, and tumor necrosis; each assigned a quantitative value, with the sum of these three values used to determine grade. A three-scale system would provide two separate groups in our study, as only high and intermediate grade sarcomas will be evaluated. 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. See 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
10.2.4 **Histopathological Assessment**

- 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 specimen criteria, storage, and shipping)

10.3.1 Biologic studies will be performed on the paraffin sections including:

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

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

10.3.1.3 Serum and 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 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: one prior to receiving regularly scheduled SU5416 dose and one within 1 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); and 5) See Appendix X Patients with positive margins will have one additional serum/plasma VEGF and bFGF level determined 3-4 months following completion of post-operative 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 (both serum and plasma). 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 will be analyzed pre-, during, 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 specimens submitted to LDS Hospital. See Section 10.1.1.

11.0 **PATIENT ASSESSMENTS**

11.1 **Study Parameters**

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<th>Pre-Entry</th>
<th>Every Week During RT</th>
<th>Prior to Each MAID Cycle</th>
<th>Every Week During MAID</th>
<th>Prior to Surgery</th>
<th>Post RX 6 Weeks</th>
<th>F/U 2 Years Post Therapy, Every 3 Months</th>
<th>F/U Years 2-5, Every 6 months</th>
<th>F/U &gt;5 Years Post Therapy, Yearly</th>
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</table>

a. Conventional CT and MRI 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. 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 guidelines for optional dynamic MRI.
b. Patients with history of myocardial infarction (MI) may participate in study if they are greater than 6 months post MI and have radiologic evidence of an ejection fraction of ≥ 50%. Ejection fraction may be assessed with MUGA scan or echocardiography.
c. Repeat if clinically indicated
d. To be performed in all females with child-bearing potential
e. Every 6 mos following treatment
f. Twice weekly during G-CSF administration
g. Blood draw within 1 hour of first SU5416 infusion; 1-2 weeks prior to surgery, need 2 samples: prior to receiving scheduled SU5416 dose and within 1 hour of completion of SU5416 infusion
h. VEGF and bFGF in serum and plasma measured at initial 3 month F/U ONLY.
i. Optional, institution specific

### 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.

**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).*

**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).*

**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.

**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.

**Record of Timing of Therapies**

Modification of chemotherapy dose associated with toxicity is noted in Section 7.8. Delays in institution of chemotherapy, radiation therapy or surgery should be recorded. We anticipate 21 days between cycles
of chemotherapy. Ideally there will be 2 days between the completion of chemotherapy and the institution of the pre-operative radiation doses. Surgery should be completed by day 80 of protocol \(\textit{time from first chemotherapy to time of surgery}\). Delay from day 80 to 94 will be considered a minor variation. Delay beyond day 94 will be considered a major deviation. Chemotherapy should be instituted between 21 days and 35 days following surgery. Delay beyond 35 days following surgery 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.

*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 institution of post-operative chemotherapy (see Section 11.2.2).

11.3.2 **Category 2**: This represents a more serious problem which seriously delays the institution of adjuvant chemotherapy. 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.

11.4 **Definition of Recurrence**

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

11.5 **Time to Recurrence**

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

11.6 **Survival**

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

11.7 **Follow-up**

Patients will be followed until death. Followup 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.

11.8 **Withdrawal from the Study**

11.8.1 Criteria for withdrawal from protocol treatment include but are not limited to unacceptable toxicity, progressive disease, and patient wish to withdraw unilaterally. Follow up will continue on all patients that are removed from protocol therapy. Decision to withdraw from study will not affect future treatment or treatment options.

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. All patients will undergo initial conventional and dynamic MR imaging within 4 weeks of start of treatment. To assess patient’s response to neoadjuvant chemotherapy, angiogenesis inhibitor SU5416, and radiotherapy, conventional and dynamic MR imaging will be performed pre and post-treatment [preoperatively] (See Section 11.0).

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 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 \(\text{(TR/TE, 500-600/10-15 with two excitations and a 256 x 256 matrix)}\) will be followed by transverse fast spin-echo T2 weighted images \(\text{(TR/TR, 3500-4000/80-90 with one excitation and a 256 x 256 matrix)}\). The choice of coil and field of view \(\text{(20-40)}\) will vary depending on the location of the lesion. The section thickness will range between 4-10 mm. with a 0.5 to 2-mm intersection gap. All imaging parameters will be recorded on a report form (See Appendix XIII). 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.

11.9.5 A standard dose of gadopentetate dimeglumine (0.1 mmoI 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.

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

11.9.7 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 indeterminant, and the type of involvement further classified as invasion or displacement.

11.10 Guidelines for tagged red-blood cell SPECT scanning for Musculoskeletal Sarcomas (Optional, Selected Institutions)

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 microvascularity 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 pre-operative 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 the variation of red cell accumulation within the tumor before and after pre-operative combined modality therapy. 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 LucUrbain: FAX # (215) 379-5717; office phone (215) 728-3041, e-mail: JL_Urbain@fccc.edu.

11.10.6 Data Collection Sheet: See Appendix VI.
Post-Induction Evaluation Form (F0)  Mailed within 48 hours of the re-evaluation before surgery

Specimen Transmittal (ST)  On day of surgery, pre-op specimen

Surgery Form (S1)  Within two weeks post surgery
Operative Notes (S2)
Surgical Pathology Report (S5)
Pathology Slides/Blocks (P2)
Peri-operative Questionnaire (PO)  4 weeks post surgery

Specimen Transmittal (ST)  6 weeks post surgery

Radiotherapy Form (T1)  Within one week of end of post surgical RT if given

Follow-up Form (F1)  At 6, 9, and 12 months from treatment start; q 4 months x 1 year, q 6 months x 3 years, then annually. Also at progression/relapse and at death

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 Endpoints

The following are the study endpoints. They will be analyzed in detail at the completion of this study.

13.1.1 Dose-limiting toxicities (DLT) using the common toxicity criteria (CTC) will be defined as one of the following:
   a) Grade 3 or greater 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 Disease-free survival

13.1.5 Tumor markers

13.1.6 Local recurrence, distant metastases, and overall survival rates.

13.2 Overview

The goal of this study is to first establish the maximum tolerated dose (MTD) for SU5416 added to MAID followed by radiation therapy at which 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 it is suspended until a decision about dose escalation can be made, can take some time. For this reason, a maximum of 6 patients will be accrued to the current dose level, but toxicity will be evaluated in cohorts of 3 patients. However, if the evaluation of the first 3 patients is not complete once 6 patients have been entered on the current dose level, accrual will be suspended to complete the evaluation. 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 contacted immediately to lower the dose. Once the MTD for SU5416 is established, additional patients will be entered to allow for a historical comparison with RTOG 95-14.
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. Toxicity will be evaluated in cohorts of 3 patients. Initially, toxicity will be evaluated in the first 3 patients. If 0/3 patients have dose limiting toxicity (DLT), then the current dose level will be considered acceptable. If 1/3 patients have DLT, then the next 3 patients 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. If a dose level is considered unacceptable, the next lower dose that has been determined to be acceptable will be considered the MTD. At a given dose level, the probability of halting dose escalation when the true toxicity is 50% or 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 of the study is projected to take 18 months if all 3 dose levels are evaluated. The total number of patients enrolled in the phase I component will be between 6 and 18, depending on the number of dose levels evaluated.

13.3.4 Analysis Plan

13.3.4.1 Interim Reports
Interim reports will be prepared every six months in the RTOG premeeting book until MTD 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 MTD Dose
The analysis will be undertaken shortly after the MTD dose 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
The second component of this study is to estimate the two-year disease-free survival rate with the MTD dose of SU5416 added to the regimen of RTOG 95-14. In that trial, the estimated two-year rate is 73%. With 55 evaluable patients on the MTD dose, the statistical power to detect an absolute 15% improvement in the two-year disease-free survival rate as compared to RTOG 95-14 is 80% using a 0.05 significance level and a one-sided test. If an additional 10% of the sample is added to guard against ineligible or inevaluable (no data) cases, then the total number of patients enrolled will be 62 patients.

13.4.2 Patient Accrual
The accrual target is 62 patients for the phase II component. Patients treated on the MTD dose in the phase I component will be counted against this target. The patient accrual rate is projected to increase to 2.0 patients per month during the phase II component of the study. Patient accrual will continue until 62 patients are entered. It is estimated that 6 patients will be treated at the MTD in the phase I component, with 56 additional patients entered in the phase II component. Estimated accrual to the entire study is 74 patients.

13.4.3 Analysis Plan

13.4.3.1 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. It is anticipated that data collected from tissue specimens made available from RTOG 95-14 will be analyzed and made available at the completion of the phase I portion of this study (tumor-associated microvessel density and VEGF/bFGF expression, tumor cell proliferation and apoptotic rate, endothelial cell proliferation and apoptotic rate).

13.4.3.2 Analysis for 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. It is anticipated that surrogate biomarker data collected from all tissue specimens from this study will be analyzed and made available at the completion of the phase II portion of this study (serum/plasma VEGF and bFGF, tumor-associated microvessel density and VEGF/bFGF expression, tumor cell proliferation and apoptotic rate, endothelial cell proliferation and apoptotic rate).

13.5 Inclusion of Women and Minorities

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 to establish the MTD dose of SU5416 added to RTOG 95-14 protocol under the assumption of the same tolerance and efficacy across the genders and across the races. A statistical analysis will be performed to examine the possible difference between the genders and among the races. The following table gives the projected percent of patients in each race and gender group based upon RTOG 95-14 instead of projected number of patients because of the phase I component.

**Projected Gender and Minority Inclusion**

<table>
<thead>
<tr>
<th></th>
<th>American Indian or Alaskan Native</th>
<th>Asian</th>
<th>Black or African American</th>
<th>Hispanic or Latino</th>
<th>Native Hawaiian or Pacific Islander</th>
<th>White</th>
<th>Other or Unknown</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>0</td>
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<td>0</td>
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<td>13</td>
<td>0</td>
<td>0</td>
<td>61</td>
<td>0</td>
<td>74</td>
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</table>
REFERENCES


APPENDIX I
RTOG S-0121

A PHASE I/II STUDY OF NEOADJUVANT CHEMOTHERAPY, ANGIogenesis INHIBITOR
SU5416 (NSC #696819; A TK INHIBITOR ANTI-ANGIOGENESIS COMPOUND), AND
RADIATION THERAPY IN THE MANAGEMENT OF HIGH-RISK, HIGH GRADE SOFT
TISSUE SARCOMAS OF THE EXTREMITIES AND BODY WALL

SAMPLE CONSENT FOR RESEARCH STUDY

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
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.

WHY IS THIS STUDY BEING DONE?

The standard treatment for patients with large soft tissue sarcomas is generally radiation,
surgery, and often chemotherapy to try to prevent the cancer from returning. The purpose of this
study is to test whether the addition of an investigational drug, Sugen 5416 (SU5416), to
chemotherapy, radiation, and surgery might be more effective in treating your cancer.

This study has two parts. In Phase I, patients will receive SU5416 in doses between 85-145
mg/m² in addition to the standard therapy in order to find out the maximum dose of SU5416 that
can be safely given. In Phase II of the study, all patients will receive chemotherapy plus the
maximum tolerated dose of SU5416 (determined in Phase I) to evaluate the effectiveness of this
treatment in treating sarcomas. Patients who have tumor cells at the edges of removed tissue
after surgery also will receive additional radiation therapy.

This research is being done because better treatments for controlling soft tissue sarcomas are
needed. Tumors and spreading cancer need a new supply of blood vessels in order to grow.
SU5416 is an experimental drug that prevented the growth of new blood vessels when tested in
laboratory and animal studies. SU5416 has undergone its first phase of clinical trials in people,
but at this time we do not know if this medicine will work against your cancer in the dose used
for this study. SU5416 is a new drug and is not yet available outside of research institutions. It
will be provided to you free of charge for this study.

HOW MANY PEOPLE WILL TAKE PART IN THE STUDY?
About 6-18 people will take part in the first phase of this study. Some of these people will be included in Phase II of the study in evaluating response to this treatment. A total of about 62 people will take part in Phase II.

**WHAT IS INVOLVED IN THE STUDY?**

You will receive three cycles of chemotherapy (once every three weeks) with the addition of SU5416, alternating with two courses of radiation therapy (RT). You will receive one of three doses of SU5416 (85, 110, or 145 mg/m²). The dose of SU5416 will be increased only after the safety at the previous dose level has been confirmed in multiple people. The day after each cycle of chemotherapy ends, you also will receive a drug, G-CSF, which may decrease the chances of infection caused by low blood counts from the other chemotherapy drugs. G-CSF will continue until your blood counts improve. Following the third cycle of chemotherapy and SU5416, you will have the whole tumor removed surgically. There will be approximately 2-1/2 months between the start of treatment and surgery. If there are any tumor cells at the edges of the removed tissue, you will have additional radiation. Regardless of whether you receive the additional radiation treatments, you will receive three more cycles of chemotherapy and SU5416 following surgery. The chemotherapy in this study will consist of mesna, adriamycin, ifosfamide, and dacarbazine.

Each cycle of chemotherapy is given over four days as an i.v. (in your vein). You may be hospitalized for each cycle. The drug SU5416 will be given twice weekly, but you do not have to be hospitalized. You will be observed for a minimum of 3 hours after the first three administrations of SU5416. Radiation is given as an outpatient. The radiation treatments take a few minutes once a day Monday through Friday over three weeks. The following table shows the planned schedule:

<table>
<thead>
<tr>
<th>TX</th>
<th>Cycle 1 Days</th>
<th>RT a Days</th>
<th>Cycle 2 Days</th>
<th>RT Days</th>
<th>Cycle 3 Days</th>
<th>Surg Day</th>
<th>Cycle 4 Days</th>
<th>Cycle 5 Days</th>
<th>Cycle 6 Days</th>
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</thead>
<tbody>
<tr>
<td>Mesna</td>
<td>1-4</td>
<td>22-25</td>
<td>43-46</td>
<td></td>
<td></td>
<td></td>
<td>101-104</td>
<td>122-125</td>
<td>143-146</td>
</tr>
<tr>
<td>Adria</td>
<td>1-3</td>
<td>22-24</td>
<td>43-45</td>
<td></td>
<td></td>
<td></td>
<td>101-103</td>
<td>122-124</td>
<td>143-145</td>
</tr>
<tr>
<td>Ifos</td>
<td>1-3</td>
<td>22-24</td>
<td>43-45</td>
<td></td>
<td></td>
<td></td>
<td>101-103</td>
<td>122-124</td>
<td>143-145</td>
</tr>
<tr>
<td>Dacarb</td>
<td>1-3</td>
<td>22-24</td>
<td>43-45</td>
<td></td>
<td></td>
<td></td>
<td>101-103</td>
<td>122-124</td>
<td>143-145</td>
</tr>
<tr>
<td>SU5416</td>
<td>twice weekly b</td>
<td>twice weekly b</td>
<td>twice weekly stop 2 days prior to surgery w</td>
<td>twice weekly b</td>
<td>twice weekly b</td>
<td>twice weekly until chemotherapy stops b</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>RT</th>
<th>7-20</th>
<th>28-41</th>
<th>d</th>
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</thead>
<tbody>
<tr>
<td>Surgery</td>
<td></td>
<td></td>
<td>80</td>
</tr>
<tr>
<td>G-CSF</td>
<td>At end of Cycle 1</td>
<td>At end of Cycle 2</td>
<td>At end of Cycle 3</td>
</tr>
</tbody>
</table>

33
a. 11 treatments in 13-15 days
b. SU5416 administered pre-operative chemotherapy and radiation therapy, as well as with post-operative chemotherapy
c. SU5416 administered as a single agent between completion of cycle 3 (Day 46) until within 2-5 days of surgery (Day 75-78)
d. For patients with positive margins, 16 Gy (2 Gy x 8 fractions) will be given by external beam (Day 94, assuming wound healing is good)

Before beginning the study, you will have the following tests and procedures to determine if you are eligible. These are routine tests and procedures. Depending on the last time you had these tests performed, you may need to have some or all of them repeated prior to entering this study.

- Physical exam (including vital signs and a review of your history)
- Blood and urine tests
- Electrocardiogram (a test to check your heart)
- X-rays of the area of the tumor
- MRI or CT scan (tests to measure tumor size and shape)

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

- You will be seen by a doctor for a physical examination every week.
- You will have standard blood tests to evaluate your blood counts, liver and kidney function every week that you are on the trial.
- You will have MRI or CT scans to measure the size of your tumor before beginning the study, prior to surgery, and six weeks after radiation therapy. The CT and MRI scans will also be repeated every six months following treatment.
- You may have a nuclear medicine scan called a tagged red-blood cell scan before and after treatment to measure tumor blood flow. You may have dye injected into your vein during the red-blood cell scan, which can occasionally cause an allergic reaction. Your doctor will discuss these risks with you at the time of the scan. The amount of radiation you receive from the red-blood cell scanning is very small.
- Many of these tests will be repeated during the study. If you participate in this study, some of these tests may be done more frequently than if you were not taking part in the study.
- A small amount of your blood will be drawn before treatment, during treatment, at the end of treatment before surgery, and 6 weeks after surgery, to be used for research purposes. These studies will evaluate the effect of SU5416 on the tumor blood vessels. You will not be billed for tests performed for research purposes during this study.
- You will be given medicines to block possible allergic reactions before the SU5416. You will be in the clinic for about 3 hours on each occasion. In addition, you will be observed for 3 hours in the clinic after the first three treatments with SU5416.
- As part of this protocol you will be followed at least every three months for two years, though it may be more frequent if necessary. Later in the study, you will be followed every 6 months. Beyond 5 years you will be followed yearly. If your disease begins to grow despite treatment with SU5416, or if the drug causes unacceptable side effects, the treatment will be stopped. Your doctor will discuss alternative plans for continued care with you at that time. If you stop treatment on this study, your doctor will continue to collect general information about your health status, and any other treatments you may receive, every 3 months.
Also, at the time of your diagnosis by biopsy, all or 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?**

You will have chemotherapy, radiation therapy, and will receive Sugen for approximately eleven weeks, followed by surgery. About 2-3 weeks after surgery, you will receive chemotherapy and Sugen (and possibly radiation therapy) for approximately 6 weeks.

Follow-up visits will take place every 3 months for the first 2 years after treatment, then every 6 months during the third through fifth years, then annually for the rest of your life. The investigator and/or your doctor may decide to take you off the study if it is in your best interest medically.

You can stop participating at any time. Your decision to withdraw from the study will not affect in any way your medical care and/or benefits. If you decide to stop participating in the study, we encourage you to discuss your decision with your doctor.

**WHAT ARE THE RISKS OF THE STUDY?**

While on the study, you are at risk for the side effects described below. You should discuss these with your doctor and/or with a member of the research study team. There may also be other side effects that we cannot predict. You may receive other drugs to make side effects less serious and make you more comfortable. Many side effects go away shortly after treatment is stopped, but in some cases side effects can be serious or long lasting, or even permanent.

**Chemotherapy**

*Very Likely*

- Loss of hair
- Nausea and/or vomiting
- Loss of appetite
- Decreased blood counts that may increase the chance of infection or bleeding

**Mesna**

*Very Likely*

- A bad taste in your mouth
• Diarrhea
• Nausea and/or vomiting
• Abdominal pain
• Headache
• Tiredness
• Rash
• Joint and muscle pain

Less Likely, but Serious
• Low blood pressure
• Allergic reactions

Adriamycin
Very Likely
• Nausea and/or vomiting
• Mouth sores
• Diarrhea
• Hair loss
• Discoloration of the nails, skin, and urine
• Fever
• Rapid heart beat
• Decreased blood counts, which could lead to an increased risk of infection, weakness, or bleeding complications. You might need antibiotics, hospitalization, and/or transfusions if these problems are severe.
• If some of the drug accidentally leaks out of the vein where it is injected, severe irritation and ulceration of the skin and soft tissues can occur.
• With prolonged usage, there is a risk of heart damage, which might be permanent. Symptoms of heart damage include shortness of breath, decreased exercise tolerance, and swollen ankles.

Ifosfamide
Very Likely
• Nausea and/or vomiting
• Loss of appetite
• Mouth sores
• Constipation and/or diarrhea
• Hair loss
• Rash and/or itching
• Blood in the urine
• Drowsiness
• Dizziness
• Confusion
• Vein inflammation
• Abnormalities of liver and kidney blood tests, which usually do not lead to significant health problems
• Decreased blood counts, which could lead to an increased risk of infection, weakness and fatigue, or bleeding complications. You might need antibiotics, hospitalization, and/or transfusions if these problems are severe.
• Low or high blood pressure

**Dacarbazine**

*Very Likely*
• "Flu-like" symptoms of fever, severe nausea and vomiting, chills, and tiredness
• If some of the drug accidentally leaks out of the vein where it is injected, severe irritation and ulceration of the skin and soft tissues can occur.
• Decreased blood counts which could lead to an increased risk of infection, weakness and fatigue, or bleeding complications

*Less Likely*
• A metallic taste in the mouth
• Hair loss
• Prickling or tingling of the face
• Sensitivity to light
• Abnormalities of liver and kidney blood tests
• Allergic reactions

**G-CSF**

*Very Likely*
• Redness, swelling, itching, and pain at the injection site
• Mild to moderate muscle/bone aching, which is usually relieved with mild medication such as acetaminophen

*Rare*
• Allergic reactions

**Sugen 5416**

*Very Likely*
• Pain and burning at the site of the injection
• Headache
• Nausea and/or vomiting
• Diarrhea
• Change in bowel habits
• Change in urine color (bright yellow or orange)
• Dry and raspy voice
• Sore throat and/or cough
• Body aches and/or fever
• Dizziness
• Loss of appetite
• Mild abdominal pain
• Weight loss and/or loss of muscle mass with associated tiredness or weakness
Less Likely, but Serious

- Generalized skin rash and swelling
- Low blood pressure
- Shortness of breath
- Blood clots in the legs, lungs, brain, or liver
- Heart attack
- Kidney or liver insufficiency
- An allergic reaction has been reported in a few patients receiving drugs similar to Sugen 5416. Also, the solution in which SU5416 is mixed has been known to cause allergic reactions including rash, sweating, flushing, swelling of the skin, itching, change in heart rate, difficulty breathing, low blood pressure and abdominal, back, arm or leg pain. These reactions are sometimes severe or life threatening. Medicines are available to prevent such reactions, and you will be given these medicines before receiving SU5416. You may require hospitalization if the allergic reaction becomes severe or life threatening, and you would be removed from the study immediately.

Radiation Therapy (RT)

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.

Early RT reactions

Very Likely

- Mild (slight redness) to severe (painful skin blistering) skin reactions; may become most noticeable during chemotherapy
- Lining of the mouth and throat may become sore and red, causing difficulty swallowing (if head and neck are treated)
- Tiredness
- Reduction in blood counts, possibly resulting in bleeding or infection
- Diarrhea (if abdominal wall is treated)
- Wound healing delay after surgery

Late RT reactions

Very Likely

- Skin in the treated area may appear tanned and may stay this way for a number of years after radiation.
- Tissues in the treated area may feel hard and woody; If this occurs, it is likely to be permanent.
- Pain in a treated limb; This symptom may occur one to several years after completion of treatment and may last for many years.
- Swelling; This may occur in the first year after treatment. In many patients this will go away. Some patients will have persistent swelling and will need to use elastic stockings.
If severe, you may require the use of a pump that pushes swelling out of the extremity. Some patients will notice temporary swelling after strenuous activity.

- Bones more susceptible to fracture
- Irradiated skin, especially over the shin and elbow, may heal more slowly if injured or bruised.

**Less Likely, but Serious**

- Injury to the bowel *(if abdominal wall is treated)*
- If heart, lung, liver, or stomach are in the field of treatment, these organs could be damaged

**Rare**

- Injury to the spinal cord *(if the back area is treated)*
- Radiation can cause tumors in the irradiated tissues. This is rare *(1 in 2,000)* in adults but can occur many years after treatment.

**Surgery**

Complications may occur when tumors are removed from the legs, arms, and body wall whether or not radiation or chemotherapy is given. While surgical treatment of these tumors results in wound healing delay or infection, the addition of radiation or radiation and chemotherapy may increase this problem. Ultimately, most patients will heal satisfactorily. Risks which may be associated with surgical procedures in this study are described below:

**Patients with tumors of arm or legs**

**Likely**

- Decreased function of affected limb because of muscle, nerve, or skin damage

**Less Likely, but Serious**

- Treatment of large tumors with radiation and surgery or radiation, chemotherapy, and surgery may result in infection or lack of healing which could result in prolonged hospitalization and rarely, amputation.

**Patients with tumors of the abdominal wall**

Radiation and surgery or radiation, chemotherapy, and surgery for tumors of the abdominal wall may result in failure of the wound to heal and occasionally the development of a hernia. If the removal of the abdominal wall sarcoma is very large, it may be necessary to replace the abdominal wall with a plastic material. While this standard surgical procedure usually works well to reinforce or reconstruct body tissues, it may result in wound infection and prolonged hospitalization.

**Patients with tumors of the chest wall**

- Removal of tumors that involve the chest wall require removal of at least part of the bones that are part of the chest. To repair this, a standard surgical procedure involving the use of plastic material to reinforce or reconstruct body tissues may be required. Again, this may uncommonly result in a severe infection. Should this repair break
down, the lung could be open to the air. Other methods of repair would then be needed.

- With any operation, there is always the risk of complications related to associated heart disease, lung disease, diabetes etc. Pre-existing problems such as these may place you at increased risk for having heart or lung problems during surgery. Rarely, these complications may result in death.

**Reproductive Risks**
Because the drugs in this study can affect an unborn baby, you should not become pregnant or father a baby while on this study. For this reason, you also are not eligible to participate in this study if you are pregnant. If you have an infant, you should not nurse your baby while on this study. If you have any questions about the reproductive issues or about preventing pregnancy, please discuss them with your doctor or a member of the Study Team.

Your physician will be checking you closely to see if any of these side effects are occurring. Routine blood tests will be done to monitor the effects of treatment. Side effects usually disappear after the treatment is stopped. In the meantime, your doctor may prescribe medication to keep these side effects under control. The use of medication to help control side effects could result in added costs. This institution is not financially responsible for treatments of side effects caused by the study treatment.

**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. The addition of SU5614 to chemotherapy, radiation, and surgery may be more effective in treating your cancer, but that benefit cannot be guaranteed. 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. Please talk to your regular doctor about these and other options.
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 of the National Cancer Institute will provide you with the investigational agent, Sugen 5416, free of charge for this study. Every effort has been made to ensure adequate supplies of this investigational agent, free of charge, for all participants. If, however, SU5416 becomes commercially available while you are being treated, there is a possibility that you would be asked to purchase subsequent supplies.

You will receive no payment for taking part in this study; however, the drug G-CSF will be provided to you at no cost by the drug company. If this free G-CSF is not available for the length of the study, you or your insurance company will be charged for 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.

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.

A Data Safety and Monitoring Board, an independent group of experts, may be reviewing the data from this research throughout the study. We will tell you about the new information from this or other studies 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:

<table>
<thead>
<tr>
<th>Name</th>
<th>Telephone Number</th>
</tr>
</thead>
</table>

For information about this study, you may contact:

<table>
<thead>
<tr>
<th>Name</th>
<th>Telephone Number</th>
</tr>
</thead>
</table>

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*

<table>
<thead>
<tr>
<th>Name</th>
<th>Telephone Number</th>
</tr>
</thead>
</table>

**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

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-0121)*

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).

<table>
<thead>
<tr>
<th>New York Heart Association Functional Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Class I</td>
</tr>
<tr>
<td>Class II</td>
</tr>
<tr>
<td>Class III</td>
</tr>
<tr>
<td>Class IV</td>
</tr>
</tbody>
</table>

44
APPENDIX III - A

STAGING SYSTEM (AJCC, 5th edition - 1998)

DEFINITION OF TNM

Primary Tumor (T)

<table>
<thead>
<tr>
<th>Code</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>TX</td>
<td>Primary tumor cannot be assessed</td>
</tr>
<tr>
<td>T0</td>
<td>No evidence of primary tumor</td>
</tr>
<tr>
<td>T1</td>
<td>Tumor 5 cm or less in greatest dimension</td>
</tr>
<tr>
<td>T1a</td>
<td>Superficial</td>
</tr>
<tr>
<td>T1b</td>
<td>Deep</td>
</tr>
<tr>
<td>T2</td>
<td>Tumor more than 5 cm in greatest dimension</td>
</tr>
<tr>
<td>T2a</td>
<td>Superficial</td>
</tr>
<tr>
<td>T2b</td>
<td>Deep</td>
</tr>
</tbody>
</table>

Regional Lymph Nodes (N)

<table>
<thead>
<tr>
<th>Code</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>NX</td>
<td>Regional lymph nodes cannot be assessed</td>
</tr>
<tr>
<td>N0</td>
<td>No regional lymph node metastasis</td>
</tr>
<tr>
<td>N1</td>
<td>Regional lymph metastasis</td>
</tr>
</tbody>
</table>

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)

<table>
<thead>
<tr>
<th>Code</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>MX</td>
<td>Presence of distant metastasis cannot be assessed</td>
</tr>
<tr>
<td>M0</td>
<td>No distant metastasis</td>
</tr>
<tr>
<td>M1</td>
<td>Distant metastasis</td>
</tr>
</tbody>
</table>

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 IB: G1-2 T2a N0 N0 M0
Stage IIA  G1-2  T2b  N0  N0  M0
Stage IIB  G3-4  T1a-1b  N0  M0
Stage IIC  G3-4  T2a  N0  N0  M0
Stage III G3-4  T2b  N0  N0  M0
Stage IV Any G  Any T  N1  M0
             Any G  Any T  N0  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
### Enneking System for Staging of Sarcomas of Soft Tissues or Bone

<table>
<thead>
<tr>
<th>Stage</th>
<th>Characteristic</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Low Grade</td>
</tr>
<tr>
<td>IA</td>
<td>Intracompartmental</td>
</tr>
<tr>
<td>IB</td>
<td>Extracompartmental</td>
</tr>
<tr>
<td>II</td>
<td>High Grade</td>
</tr>
<tr>
<td>IIA</td>
<td>Intracompartmental</td>
</tr>
<tr>
<td>IIB</td>
<td>Extracompartmental</td>
</tr>
<tr>
<td>III</td>
<td>Any Grade</td>
</tr>
<tr>
<td></td>
<td>N1 or M1</td>
</tr>
</tbody>
</table>
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.

b. 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.

c. 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.

d. The Principal Investigator will also report the details of the significant reaction to the Study Chairman by telephone.

e. 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).

f. 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.

g. 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.

h. The Principal Investigator, when participating in RTOG-coordinated Intergroup studies, is obligated to comply with all additional reporting specifications required by an individual study.

i. Institutions must also comply with their individual Institutional Review Board policy with regard to toxicity reporting procedure.

j. Failure to comply with reporting requirements in a timely manner may result in suspension of patient registration.

k. RADIATION TOXICITY GUIDELINES

l. 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.
m. 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.

n. Appropriate data forms, and if requested a written report, must be submitted to Headquarters within 10 working days of the telephone report.

o. **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**

p. 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.

q. Unknown adverse reactions (> 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.

r. All neurotoxicities (> grade 3) from radiosensitizer or protector drugs are to be reported within 24 hours by phone to RTOG Headquarters and to the Study Chairman.

s. 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
1. **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.**

u. **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. **A written report may be required.**

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

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 ___________________________________________
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

<table>
<thead>
<tr>
<th>Baseline</th>
<th>Nadir</th>
<th>Latest Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Date/Value</td>
<td>Date/Value</td>
<td>Date/Value</td>
</tr>
<tr>
<td>WBC or PMN</td>
<td>/</td>
<td>/</td>
</tr>
<tr>
<td>Platelets</td>
<td>/</td>
<td>/</td>
</tr>
<tr>
<td>Hgb or Hct</td>
<td>/</td>
<td>/</td>
</tr>
</tbody>
</table>

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.)

<table>
<thead>
<tr>
<th>IND</th>
<th>Non-IND</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drug</td>
<td>Drug</td>
</tr>
<tr>
<td>Unrelated</td>
<td>_____</td>
</tr>
<tr>
<td>Unlikely</td>
<td>_____</td>
</tr>
<tr>
<td>Possible</td>
<td>_____</td>
</tr>
<tr>
<td>Probable</td>
<td>_____</td>
</tr>
<tr>
<td>Definite</td>
<td>_____</td>
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</table>

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

<table>
<thead>
<tr>
<th>PATIENT ID#</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Date of Study</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| Dose Infiltration (circle) |  |  |  |  |
|----------------------------|  |  |  |  |
| PRE-TREATMENT | POST-TREATMENT |  |  |  |
| Yes | No | Yes | No |  |  |  |  |

| 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 angiogenesis. Numerous studies indicate that MVD associated with primary solid tumors correlates with increased metastasis or recurrence, but its utility as a prognostic indicator in this disease remains in question.

SPECIFIC AIMS

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

2. Determine baseline (prior to initiation of any therapy) angiogenic response to cancer.
3. Evaluate changes in tumor-associated angiogenesis as a biomarker of response following specific treatment modality (external beam irradiation, SU5416, or combination therapy).
4. 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. SU5416 significantly decreases vascularity associated with neurogenic sarcoma xenografts in preclinical animal models. 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.

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 Back (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.

```markdown
<table>
<thead>
<tr>
<th>RTOG Pathology Submission Form</th>
</tr>
</thead>
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<tr>
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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. 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 dianaminobenzidine (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 interface. 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

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

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.

________________________


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.


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.

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:

5. 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).

6. 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 and as a vascular permeability factor, it is produced by a large variety of human tumors. 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. Expression of VEGF by invasive tumors has been shown to correlate with vascularity, cellular proliferation and the ability to metastasize of several human cancers. We have reported quantitative immunohistochemical analysis of VEGF in human tumor xenograft models. 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) and Flt-1 (fms-like tyrosine kinase). In situ hybridization has shown that KDR and Flt-1 are expressed exclusively on endothelial cells. KDR is involved in VEGF-induced mitogenesis, but the role of Flt-1 is not clear. 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. 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 gene. 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\textsuperscript{xx}, the growth of human gliomas\textsuperscript{xxi}, and correlates with human hepatic tumorigenesis\textsuperscript{xxii}. 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\textsuperscript{xxiii,xxiv}. SU5416 significantly decreases vascularity associated with neurogenic sarcoma xenografts in preclinical animal models\textsuperscript{xxv}.

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\textsuperscript{xxvi}. 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 \textit{in vivo} by neutralizing antibodies to FGF-2 (bFGF)\textsuperscript{xxvii}. We have shown that angiogenic endothelium expresses mRNA for both bFGF and its receptor\textsuperscript{xxviii}. Interestingly, VEGF and bFGF have been shown to synergize using in vivo angiogenesis assays\textsuperscript{xxix}, 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\textsuperscript{xl,xli}. VEGF and bFGF act as major tumor-produced regulators capable of promoting the angiogenic switch\textsuperscript{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\textsuperscript{xxxii,xxxiii,xxxiv,xxxv}.

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

61
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. 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 polyclonal antibody (Santa Cruz Cat. # sc-507), goat anti-FGF-2 polyclonal antibody (Santa Cruz Cat. # sc-79), mouse anti-human Flk-1 monoclonal antibody (Santa Cruz Cat. # sc-6251), rabbit anti-Flt-1 polyclonal antibody (Santa Cruz Cat. # sc-9029), or mouse anti-human Bek 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.

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. 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/Autocyt, 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

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 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.
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. Evaluation of both proliferation and apoptotic indices within a neoplastic lesion has been utilized to evaluate the efficacy in cancer therapeutics. 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. This effect is associated with a reduction in tumor cell proliferation and an increase in tumor cell apoptosis. Immunohistochemical quantitiation 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. 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. 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 dianaminobenzidine (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 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.


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 basic fibroblast growth factor (bFGF) have been identified as powerful promoters of tumor-induced angiogenesis\textsuperscript{lviii,lix}. Both growth factors can be detected in human body fluids by specific enzyme-linked immunosorbent assays\textsuperscript{lx,lxi}, and elevated serum concentrations of each are detectable in patients with various types of tumor, including soft tissue sarcomas\textsuperscript{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\textsuperscript{lvx}. 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\textsuperscript{lix}. Circulating angiogenic factors can be measured from either serum or plasma. Practically all circulating VEGF is carried within the platelets and the leukocytes\textsuperscript{lx}. Since VEGF measured from serum has its origin in the blood cells, hemolysis or platelet activation may result in liberation of VEGF into serum\textsuperscript{lxvi}. However, platelet-derived VEGF is suggested to reflect the biology of cancer cells due to implications that platelet-tumor interactions may occur during metastasis\textsuperscript{lxvii}. 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), 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)
   - 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 µl)
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, RT/CT in Patients with High Grade Soft Tissue Sarcomas**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Priority</th>
<th>Purpose and Proposed Techniques</th>
<th>Quantity</th>
<th>Pre-Treatment</th>
<th>On-Treatment/Pre-Resection</th>
<th>Post-Treatment/At Resection</th>
<th>Post-Resection</th>
<th>Responsible Investigator</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Baseline</td>
<td>1-2 wks prior to resection</td>
<td>Day 0 (resection)</td>
<td>3-6 wks at F/U</td>
<td></td>
</tr>
</tbody>
</table>
| Surgical Pathology:  
Tumor-stroma interface | 1 | Immunohistochemistry  
4. TUNEL assay  
5. Paraffin-embedded Tissue Block | X⁷ | NA | X | NA | Dr. Watson |
| Blood⁸ - peripheral | 2 | ELISA³ | X¹⁰ | X¹¹ | X | X | |
| Surgical Pathology:  
Biopsy scar | 3 | Immunohistochemistry  
13. Paraffin-embedded Tissue Block | NA | NA | X | NA | Dr. Watson |

¹All samples obtained with tumor in situ prior to initiating treatment.

²Following 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.

⁴Immunohistochemistry 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 open biopsy is encouraged in order to obtain tissue for baseline examination; multiple core biopsy samples under CT guidance are acceptable, but 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 lst 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.
APPENDIX XII

Response Evaluation Criteria in Solid Tumors (RECIST) Quick Reference

Eligibility

- Only patients with measurable disease at baseline should be included in protocols where objective tumor response is the primary endpoint.

**Measurable disease** - the presence of at least one measurable lesion. If the measurable disease is restricted to a solitary lesion, its neoplastic nature should be confirmed by cytology/histology.

**Measurable lesions** - lesions that can be accurately measured in at least one dimension with longest diameter \( \geq 20 \text{ mm} \) using conventional techniques or \( \geq 10 \text{ mm} \) with spiral CT scan.

**Non-measurable lesions** - all other lesions, including small lesions (longest diameter <20 mm with conventional techniques or <10 mm with spiral CT scan), i.e., bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusion, inflammatory breast disease, lymphangitis cutis/pulmonis, cystic lesions, and also abdominal masses that are not confirmed and followed by imaging techniques; and.

- 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 and never more than 4 weeks before the beginning of the 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.

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

Methods of Measurement –

- CT and MRI are the best currently available and reproducible methods to measure target lesions selected for response assessment. Conventional CT and MRI 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. Head and neck tumors and those of extremities usually require specific protocols.

- Lesions on chest X-ray are acceptable as measurable lesions when they are clearly defined and surrounded by aerated lung. However, CT is preferable.

- When the primary endpoint of the study is objective response evaluation, ultrasound (US) should not be used to measure tumor lesions. It is, however, a possible alternative to clinical measurements of superficial palpable lymph nodes, subcutaneous lesions and thyroid nodules. US might also be useful to confirm the complete disappearance of superficial lesions usually assessed by clinical examination.

- The utilization of endoscopy and laparoscopy for objective tumor evaluation has not yet been fully and widely validated. Their uses in this specific context require sophisticated equipment and a high level of expertise that may only be available in some centers. Therefore, the utilization of such techniques for objective tumor response should be restricted to validation purposes in specialized centers. However, such techniques can be useful in confirming complete pathological response when biopsies are obtained.

- Tumor markers alone cannot be used to assess response. If markers are initially above the upper normal limit, they must normalize for a patient to be considered in complete clinical response when all lesions have disappeared.

Version 2, May 2000
Cytology and histology can be used to differentiate between PR and CR in rare cases (e.g., after treatment to differentiate between residual benign lesions and residual malignant lesions in tumor types such as germ cell tumors).

**Baseline documentation of “Target” and “Non-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.

- All other lesions (or sites of disease) should be identified as *non-target lesions* and should also be recorded at baseline. Measurements of these lesions are not required, but the presence or absence of each should be noted throughout follow-up.

**Response Criteria**

**Evaluation of target lesions**

* Complete Response (CR): Disappearance of all target lesions

* Partial Response (PR): At least a 30% decrease in the sum of the LD of target lesions, taking as reference the baseline sum LD

* 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

* 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

**Evaluation of non-target lesions**

* Complete Response (CR): Disappearance of all non-target lesions and normalization of tumor marker level

* Incomplete Response/Stable Disease (SD): Persistence of one or more non-target lesion(s) or/and maintenance of tumor marker level above the normal limits

* Progressive Disease (PD): Appearance of one or more new lesions and/or unequivocal progression of existing non-target lesions

(1) Although a clear progression of “non target” lesions only is exceptional, in such circumstances, the opinion of the treating physician should prevail and the progression status should be confirmed later on by the review panel (or study chair).
Evaluation of best overall response

The best overall response is the best response recorded from the start of the treatment until disease progression/recurrence (taking as reference for PD the smallest measurements recorded since the treatment started). In general, the patient’s best response assignment will depend on the achievement of both measurement and confirmation criteria.

<table>
<thead>
<tr>
<th>Target lesions</th>
<th>Non-Target lesions</th>
<th>New Lesions</th>
<th>Overall response</th>
</tr>
</thead>
<tbody>
<tr>
<td>CR</td>
<td>CR</td>
<td>No</td>
<td>CR</td>
</tr>
<tr>
<td>CR</td>
<td>Incomplete response/SD</td>
<td>No</td>
<td>PR</td>
</tr>
<tr>
<td>PR</td>
<td>Non-PD</td>
<td>No</td>
<td>PR</td>
</tr>
<tr>
<td>SD</td>
<td>Non-PD</td>
<td>No</td>
<td>SD</td>
</tr>
<tr>
<td>PD</td>
<td>Any</td>
<td>Yes or No</td>
<td>PD</td>
</tr>
<tr>
<td>Any</td>
<td>PD</td>
<td>Yes or No</td>
<td>PD</td>
</tr>
<tr>
<td>Any</td>
<td>Any</td>
<td>Yes</td>
<td>PD</td>
</tr>
</tbody>
</table>

- Patients with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be classified as having “symptomatic deterioration”. Every effort should be made to document the objective progression even after discontinuation of treatment.

- In some circumstances it may be difficult to distinguish residual disease from normal tissue. When the evaluation of complete response depends on this determination, it is recommended that the residual lesion be investigated (fine needle aspirate/biopsy) to confirm the complete response status.

Confirmation

- The main goal of confirmation of objective response is to avoid overestimating the response rate observed. In cases where confirmation of response is not feasible, it should be made clear when reporting the outcome of such studies that the responses are not confirmed.

- To be assigned a status of PR or CR, changes in tumor measurements must be confirmed by repeat assessments that should be performed no less than 4 weeks after the criteria for response are first met. Longer intervals as determined by the study protocol may also be appropriate.

- In the case of SD, follow-up measurements must have met the SD criteria at least once after study entry at a minimum interval (in general, not less than 6-8 weeks) that is defined in the study protocol.

Duration of overall response

- The duration of overall response is measured from the time measurement criteria are met for CR or PR (whichever status is recorded first) until the first date that recurrence or PD is objectively documented, taking as reference for PD the smallest measurements recorded since the treatment started.

Version 2, May 2000
Duration of stable disease

- SD is measured from the start of the treatment until the criteria for disease progression are met, taking as reference the smallest measurements recorded since the treatment started.

- The clinical relevance of the duration of SD varies for different tumor types and grades. Therefore, it is highly recommended that the protocol specify the minimal time interval required between two measurements for determination of SD. This time interval should take into account the expected clinical benefit that such a status may bring to the population under study.

Response review

- For trials where the response rate is the primary endpoint it is strongly recommended that all responses be reviewed by an expert(s) independent of the study at the study’s completion. Simultaneous review of the patients’ files and radiological images is the best approach.

Reporting of results

- All patients included in the study must be assessed for response to treatment, even if there are major protocol treatment deviations or if they are ineligible. Each patient will be assigned one of the following categories: 1) complete response, 2) partial response, 3) stable disease, 4) progressive disease, 5) early death from malignant disease, 6) early death from toxicity, 7) early death because of other cause, or 9) unknown (not assessable, insufficient data).

- All of the patients who met the eligibility criteria should be included in the main analysis of the response rate. Patients in response categories 4-9 should be considered as failing to respond to treatment (disease progression). Thus, an incorrect treatment schedule or drug administration does not result in exclusion from the analysis of the response rate. Precise definitions for categories 4-9 will be protocol specific.

- All conclusions should be based on all eligible patients.

- Subanalyses may then be performed on the basis of a subset of patients, excluding those for whom major protocol deviations have been identified (e.g., early death due to other reasons, early discontinuation of treatment, major protocol violations, etc.). However, these subanalyses may not serve as the basis for drawing conclusions concerning treatment efficacy, and the reasons for excluding patients from the analysis should be clearly reported.

- The 95% confidence intervals should be provided.
APPENDIX XIII

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
   - ___Sagital 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 dimeglulmine 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.
APPENDIX XIV (9/12/01)

Filgrastim (G-CSF) Drug Request Form

Amgen Study No:   Group Study No: RTOG S-0121: “A Phase I/II Study of Neoadjuvant Chemotherapy, Angiogenesis Inhibitor SU5416 (NSC #696819; A TK Inhibitor Anti-Angiogenesis Compound), and Radiation Therapy In The Management of High-Risk, High-Grade, Soft Tissue Sarcomas Of The Extremities and Body Wall”

Requested by:   Ship To:

Pharmacist: ________________________________ Name: ________________________________

Institution: ________________________________ Address*: ________________________________

RTOG Study Number: __________________________ (must be included)

Principal Investigator: __________________________ * Please do not use P.O. Box numbers

Phone #: ________________________________ Fax: ________________________________

* Reminder: See protocol section on drug formulation for instructions regarding amounts of drug to order.

G-CSF will be shipped refrigerated. Orders received by 11:30 a.m. PST Monday through Thursday will be shipped for next day delivery. Orders received by 11:30 a.m. PST on Friday will be shipped for receipt the following Monday.

<table>
<thead>
<tr>
<th>Pt. ID</th>
<th>Pt Initials (Last, First)</th>
<th># of Vials*</th>
<th>RTOG Case#</th>
<th>Starter Supply (For this pt.)</th>
<th>Re-order (For this pt.)</th>
</tr>
</thead>
<tbody>
<tr>
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</tbody>
</table>

Date of Drug Request

Pharmacist Signature

Return Completed, Signed, and Dated form to:

UintaVision, Inc./Axion, Inc.
232 Castro Street, Suite #2
San Francisco, CA 94114
Fax: 650-745-3877
APPENDIX XV (9/12/01)

RETURNED MEDICATION PACKING SLIP

Institution Name: ________________________________________________________

Address: __________________________________________________________________

Principal Investigator: _____________________________________________________

Amgen Study No:    Group Study No:  RTOG S-0121
“A Phase I/II Study of Neoadjuvant Chemotherapy, Angiogenesis Inhibitor SU5416 (NSC #696819; A TK Inhibitor Anti-Angiogenesis Compound), and Radiation Therapy In The Management of High-Risk, High-Grade, Soft Tissue Sarcomas Of The Extremities and Body Wall”

Instructions:
Per FDA requirements, please retain a copy of this completed form for your files. Drug being returned for any reason should be sent, together with this original form, to UintaVision, Inc./Axion, Inc., 232 Castro Street, Suite #2, San Francisco, CA, 94114. Only drug returns are to be sent to this address, no other correspondence. Questions may be directed to (800) 370-2508, Monday through Friday 6:30 am - 1:00 pm, Pacific Standard Time. Voice Mail is available at all other times.

Study in progress? Yes  No

Drug being returned by: __________________________

Fed Ex   UPS   US Mail

Study completed per protocol? Yes  No

Date: __________________________ No. of cartons: ________

Research Associate's/Pharmacist's Signature: __________________________

Reason drug returned? (Please check one)

Drug Expired

Unused drug being returned

Return receipt requested: Yes  No

Fax number: __________________________

DESCRIPTION OF RETURN SHIPMENT

<table>
<thead>
<tr>
<th>Drug Name &amp; Vial Description</th>
<th>Lot Number</th>
<th>Number of Vials</th>
</tr>
</thead>
<tbody>
<tr>
<td>...............................</td>
<td>...............................</td>
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</tr>
</tbody>
</table>

Comments:

TO BE COMPLETED BY AMGEN

Returned shipment received on __________________________ and checked by: __________________________

(Name)


Hughes S, Hall P. The fibroblast growth factor and receptor multigene families. *J. Pathol.* 1993, **170**: 219-221.


Ferrar N, Henzel WJ. Pituitary follicular cells secrete a novel heparin-binding growth factor specific for vascular endothelial cells. *Biochem Biophys Res Commun* 1989, **161**: 851-858.


Lee JK, Hong YJ, Han CJ, Hwang DY, Hong SI. Clinical usefulness of serum and plasma vascular endothelial growth factor in cancer patients: which is the optimal specimen? *Int J Oncol* 2000, **17**: 149-152.
