Pediatric Oncology Experimental Therapeutics Investigators' Consortium (POETIC)

A Phase II Trial of Cyclophosphamide, Topotecan, and Bevacizumab (CTB) in Patients with Relapsed/Refractory Ewing's Sarcoma and Neuroblastoma

Study Drug
Bevacizumab (Avastin®)

Support Provided By
Genentech, Inc.

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Study Number POE10-01

Protocol History

Version 1.0	11/30/2011
Version 2.0	01/30/2013
Version 3.0	01/05/2016

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1.0 BACKGROUND

1.1 Disease Background

Angiogenesis, the formation of new blood vessels, is one of the key hallmarks of cancer and has been shown to be essential for tumor growth and metastasis.[1] Amongst the tumor-associated growth factors and chemokines, vascular endothelial growth factor (VEGF) family members represent a prominent group of proangiogenic factors secreted by the majority of solid tumors, leukemias and lymphomas.[2,3] The VEGF family proteins have several mechanistic properties responsible for enhancing the growth of tumors. Dvorak et al first identified this molecule as the vascular permeability factor (VPF), which resulted in the leakage of plasma proteins, growth factors, and platelets from the circulation to the tissue parenchyma in tumors and in distant organs.[4,5] For a second mechanism of action, VEGF specific receptors were found on the surface of mature endothelial cells promoting the growth of new blood vessels. Folkman et al demonstrated that endothelial cell proliferation at the tips of established blood vessels formed branching of new blood vessels to the tumor site enabling the continued growth of the primary tumor.[6] One member of this family, VEGF-A, was shown to promote new blood vessel formation by its interaction with VEGF receptor 2 (VEGFR2) on endothelial cells.[7] As an alternative explanation of new blood vessel formation, Lyden et al have shown that VEGF-A signals through two tyrosine kinase receptors, VEGFR1 and VEGFR2, expressed on bone marrow-derived stem and progenitor cells. Endothelial progenitor cells (EPCs) expressing VEGFR2 and hematopoietic progenitor cells (HPCs) expressing VEGFR1 were both required to form the earliest blood vessels (neovasculogenesis) in tumors. During early tumorigenesis, VEGF-A specifically promoted the proliferation and recruitment of $VEGFR2^+$ EPCs to the primary tumor site. Similarly, VEGF-A promoted the production and mobilization of $VEGFR1^+$ HPCs to the tumor as well. Although less well studied, $VEGFR1^+$ HPCs appear to play a critical role in regulating the interactions, such as adhesive properties, between tumor cells, endothelial cells along with the adjoining inflammatory infiltrates. $VEGFR1^+$ HPCs may also contribute to the perivascular cell network for the stabilization of blood vessels. As a third mechanism, VEGF can mobilize $VEGFR1^+$ HPCs to future sites of metastasis forming the “pre-metastatic niche” or the preconditioning necessary for a favorable microenvironment suitable for the arrival of incoming metastatic tumor cells[8]. In addition to VEGF-A, other VEGF family members, VEGF-B and PlGF (placental growth factor), can support the mobilization of $VEGFR1^+$ HPCs to the primary tumor and to the pre-metastatic sites.

Besides its paracrine effects and as a fourth mechanism, VEGF-A and its receptors, VEGFR1 and VEGFR2, form an autocrine loop on certain tumor cells enabling the direct support of certain tumor cell growth. VEGF-A and its receptors are expressed in leukemia and lymphoma as well as pediatric sarcomas and neuroblastomas [9,10]. Given the potential autocrine and paracrine effects of VEGF-A, it is not surprising that higher VEGF levels have been associated with worse prognosis in pediatric and adult patients with both liquid and solid tumors [11, 12, 13].

1.1.1 Rationale for Targeting VEGF in Pediatric Malignancies

Pediatric cancers typically have a better cure rate than the majority of adult cancers. However, refractory disease and the potential for long-term morbidity from the use of conventional therapies necessitate the development of novel treatments for children. The promise of anti-angiogenic therapy includes reduced toxicity and the potential for efficacy predominantly by targeting the tumor's blood supply. Novel agents such as bevacizumab are easily administered with little or no significant toxicity. In fact, no MTD was established in the Phase I COG study of bevacizumab in refractory pediatric solid tumors. Treatment was well tolerated with no DLTs observed. Adverse events including infusional reaction, rash, mucositis, proteinuria, and lymphopenia were Grade 1- 2 and did not meet the criteria for DLT. Increases in systolic and diastolic blood pressure not meeting CTCAE version 3 pediatric-specific criteria for hypertension were observed. There was no hemorrhage or thrombosis reported. Growth pertubation was not detected in a limited sample over the first course.[68]

The potential to prevent tumor growth and induce tumor shrinkage has been established in the adult setting as well as in pediatric tumor xenografts of neuroblastoma and sarcomas in the murine setting [14, 15]. The effect of targeting VEGF is enhanced when combined with traditional cytotoxic therapies and these findings provide evidence for combining VEGF with standard cytotoxic chemotherapeutic agents.

1.2 Bevacizumab Clinical Experience

Bevacizumab has been studied in a multitude of Phase I, II, and III clinical trials in more than 5000 patients and in multiple tumor types. In addition, data are available from 3,863 patients enrolled in two postmarketing studies in metastatic colorectal cancer (CRC). Approximately 130,000 patients have been exposed to bevacizumab as a marketed product or in clinical trials. The following discussion summarizes bevacizumab's safety profile and presents some of the efficacy results pertinent to this particular trial. Please refer to the bevacizumab Investigator Brochure for descriptions of all completed Phase I, II, and III trials reported to date.

In a large phase III study (AVF2107g) in patients with metastatic colorectal cancer, the addition of bevacizumab, a monoclonal antibody directed against vascular endothelial growth factor (VEGF), to irinotecan/5-fluorouracil/leucovorin (IFL) chemotherapy resulted in a clinically and statistically significant increase in duration of survival, with a hazard ratio of death of 0.67 (median survival 15.6 vs. 20.3 months; $p < 0.001$). Similar increases were seen in progression-free survival (6.2 vs. 10.6 months; $p < 0.001$), overall response rate (35% vs. 45%; $p < 0.01$) and duration of response (7.1 vs. 10.4 months; $p < 0.01$) for the combination arm versus the chemotherapy only arm (bevacizumab Investigator Brochure, October 2005).

Based on the survival advantage demonstrated in Study AVF2107g, bevacizumab was designated for priority review and was approved on 26 February 2004 in the United States for first-line treatment in combination with IV 5-FU–based chemotherapy for subjects with metastatic colorectal cancer.

Additional data from Phase III trials in metastatic CRC (E3200), non–small cell lung cancer (NSCLC; E4599), and metastatic breast cancer (E2100) have also demonstrated clinical benefit from bevacizumab when added to chemotherapy. In Study E3200, the

addition of bevacizumab to FOLFOX chemotherapy resulted in improved overall survival compared with FOLFOX alone (13.0 vs. 10.8 months, respectively, HR = 0.75; $p < 0.01$) in a population of previously treated CRC patients.

There was also improved overall survival in first-line NSCLC patients (E4599) treated with carboplatin/paclitaxel + bevacizumab compared with chemotherapy alone (12.3 vs. 10.3 months, respectively; HR = 0.80; $p = 0.003$). The results from this trial were the basis for FDA approval of bevacizumab for use in combination with carboplatin + paclitaxel as first-line treatment of patients with unresectable, locally advanced, recurrent or metastatic, non-squamous NSCLC in October 2006. Finally, patients with untreated metastatic breast cancer (E2100) who received bevacizumab in combination with weekly paclitaxel had a marked improvement in PFS compared with chemotherapy alone (13.3 vs. 6.7 months, respectively; HR = 0.48; $p < 0.0001$) (see the Bevacizumab Investigator Brochure for additional details).

1.2.1. Safety Profile

In the initial Phase I and II clinical trials, four potential bevacizumab-associated safety signals were identified: hypertension, proteinuria, thromboembolic events, and hemorrhage. Additional completed Phase II and Phase III studies of bevacizumab as well as spontaneous reports have further defined the safety profile of this agent. Bevacizumab-associated adverse events identified in phase III trials include congestive heart failure (CHF) primarily in metastatic breast cancer, gastrointestinal perforations, wound healing complications, and arterial thromboembolic events (ATE). These and other safety signals are described in further detail as follows and in the bevacizumab Investigator Brochure.

Hypertension: An increased incidence of hypertension has been observed in patients treated with bevacizumab. Grade 4 and 5 hypertensive events are rare. Clinical sequelae of hypertension are rare but have included hypertensive crisis, hypertensive encephalopathy, and reversible posterior leukoencephalopathy syndrome (RPLS) (Ozcan et al., 2006; Glusker et al., 2006).

There is no information on the effect of bevacizumab in patients with uncontrolled hypertension at the time of initiating bevacizumab therapy. Therefore, caution should be exercised before initiating bevacizumab therapy in these patients. Monitoring of blood pressure is recommended during bevacizumab therapy. Optimal control of blood pressure according to standard public health guidelines is recommended for patients on treatment with or without bevacizumab.

Temporary interruption of bevacizumab therapy is recommended in patients with hypertension requiring medical therapy until adequate control is achieved. If hypertension cannot be controlled with medical therapy, bevacizumab therapy should be permanently discontinued. Bevacizumab should be permanently discontinued in patients who develop hypertensive crisis or hypertensive encephalopathy.

Reproductive System And Breast Disorders: Loss of the normal functioning of the ovaries in a woman that can result in temporary or permanent menopause; the impact on fertility (temporary or permanent) is unknown.

Ovarian failure, defined as amenorrhea lasting 3 or more months with follicle-stimulating hormone (FSH) elevation (≥30 mIU/mL), was increased in patients receiving adjuvant bevacizumab plus mFOLFOX compared to mFOLFOX alone (34% vs. 2%). After discontinuation of bevacizumab, resumption of menses and an FSH level <30 mIU/mL was demonstrated in 22% (7/32) of these women. Long term effects of bevacizumab exposure on fertility are unknown. However, this risk is only applicable to female, premenopausal patients.

Proteinuria: An increased incidence of proteinuria has been observed in patients treated with bevacizumab compared with control arm patients. In the bevacizumab-containing treatment arms of clinical trials (across all indications), the incidence of proteinuria (reported as an adverse event) was up to 38% (metastatic CRC Study AVF2192g). The severity of proteinuria has ranged from asymptomatic and transient events detected on routine dipstick urinalysis to nephrotic syndrome; the majority of proteinuria events have been grade 1. NCI-CTC Grade 3 proteinuria was reported in up to 3% of bevacizumab-treated patients, and Grade 4 in up to 1.4% of bevacizumab-treated patients. The proteinuria seen in bevacizumab clinical trials was not associated with renal impairment and rarely required permanent discontinuation of bevacizumab therapy. Bevacizumab should be discontinued in patients who develop Grade 4 proteinuria (nephrotic syndrome).

Patients with a history of hypertension may be at increased risk for the development of proteinuria when treated with bevacizumab. There is evidence from the dose-finding, Phase II trials (AVF0780g, AVF0809s, and AVF0757g) suggesting that Grade 1 proteinuria may be related to bevacizumab dose.

Proteinuria will be monitored by urine protein:creatinine (UPC) ratio at least every 6 weeks. If the UPC ratio is not available, a dipstick urinalysis may be used to allow treatment to proceed.

Thromboembolic Events: Both venous and arterial thromboembolic (TE) events, ranging in severity from catheter-associated phlebitis to fatal, have been reported in patients treated with bevacizumab in the colorectal cancer trials and, to a lesser extent, in patients treated with bevacizumab in NSCLC and breast cancer trials.

Venous Thromboembolism (Including Deep Venous Thrombosis, Pulmonary Embolism, And Thrombophlebitis: In the phase III pivotal trial in metastatic CRC, there was a slightly higher rate of venous TE events in patients treated with bevacizumab plus chemotherapy compared with chemotherapy alone (19% vs. 16%).

In Study AVF2107g, a Phase III, pivotal trial in metastatic CRC, VTE events, including deep venous thrombosis, pulmonary embolism, and thrombophlebitis, occurred in 15.2% of patients receiving chemotherapy alone and 16.6% of patients receiving chemotherapy + bevacizumab.

The incidence of NCI-CTC Grade ≥ 3 venous VTE events in one NSCLC trial (E4599) was higher in the bevacizumab-containing arm compared to the chemotherapy control arm (5.6% vs. 3.2%). One event (0.2%) was fatal in the bevacizumab-containing arm; not fatal events were reported in the carboplatin/paclitaxel arm (see Bevacizumab Investigator Brochure). In metastatic CRC clinical trials, the incidence of VTE events

was similar in patients receiving chemotherapy + bevacizumab and those receiving the control chemotherapy alone.

In clinical trials across all indications the overall incidence of VTE events was 2.8%–17.3% in the bevacizumab-containing arms compared with 3.2%–15.6% in the chemotherapy control arms. The use of bevacizumab with chemotherapy does not substantially increase the risk of VTE event compared with chemotherapy alone. However, patients with metastatic CRC who receive bevacizumab and experienced a VTE event may be at higher risk for recurrence of VTE event.

Arterial Thromboembotic Events: An increased incidence of ATE events was observed in patients treated with bevacizumab compared with those receiving control treatment. ATE events include cerebrovascular accidents, myocardial infarction, transient ischemic attacks (TIAs), and other ATE events. In a pooled analysis of data from five randomized Phase II and III trials (mCRC [AVF2107g, AVF2192g, AVF0780g]; locally advanced or metastatic NSCLC [AVF0757g]; metastatic breast cancer [AVF2119g]), the incidence rate of ATE events was 3.8% (37 of 963) in patients who received chemotherapy + bevacizumab compared with 1.7% (13 of 782) in patients treated with chemotherapy alone. ATE events led to a fatal outcome in 0.8% (8 of 963) of patients treated with chemotherapy + bevacizumab and 0.5% (4 of 782) of patients treated with chemotherapy alone. Cerebrovascular accidents (including TIAs) occurred in 2.3% of patients treated with chemotherapy + bevacizumab and 0.5% of patients treated with chemotherapy alone. Myocardial infarction occurred in 1.4% of patients treated with chemotherapy + bevacizumab compared with 0.7% of patients treated with chemotherapy alone (see the Bevacizumab Investigator Brochure for additional details).

Aspirin is a standard therapy for primary and secondary prophylaxis of arterial thromboembolic events in patients at high risk of such events, and the use of aspirin ≤ 325 mg daily was allowed in the five randomized studies discussed above. Use of aspirin was assessed routinely as a baseline or concomitant medication in these trials, though safety analyses specifically regarding aspirin use were not preplanned. Due to the relatively small numbers of aspirin users and arterial thromboembolic events, retrospective analyses of the ability of aspirin to affect the risk of such events were inconclusive. However, similarly retrospective analyses suggested that the use of up to 325 mg of aspirin daily does not increase the risk of grade 1-2 or grade 3-4 bleeding events, and similar data with respect to metastatic colorectal cancer patients were presented at ASCO 2005 (Hambleton et al., 2005). Further analyses of the effects of concomitant use of bevacizumab and aspirin in colorectal and other tumor types are ongoing.

Gastrointestinal Perforation: Patients with metastatic carcinoma may be at increased risk for the development of gastrointestinal perforation and fistula when treated with bevacizumab and chemotherapy. Bevacizumab should be permanently discontinued in patients who develop gastrointestinal perforation. A causal association of intra-abdominal inflammatory processes and gastrointestinal perforation to bevacizumab treatment has not been established. Nevertheless, caution should be exercised when treating patients with intra-abdominal inflammatory processes with bevacizumab. Gastrointestinal perforation has been reported in other trials in non-colorectal cancer populations (e.g., ovarian, renal cell, pancreas, breast, and NSCLC) and may be higher in incidence in some tumor types.

Fistula: Bevacizumab use has been associated with serious cases of fistulae including events resulting in death. Fistulae in the GI tract are common (1%–10% incidence) in patients with metastatic CRC, but uncommon (0.1%–1%) or rare (0.01%–0.1%) in other indications. In addition, fistulae that involve areas of the body other than the GI tract (e.g., tracheoesophageal, bronchopleural, urogenital, biliary) have been reported uncommonly (0.1%–1%) in patients receiving bevacizumab in clinical studies and postmarketing reports. Events were reported at various time points during treatment, ranging from 1 week to > 1 year following initiation of bevacizumab, with most events occurring within the first 6 months of therapy.

Permanently discontinue bevacizumab in patients with tracheoesophageal fistulae or any Grade 4 fistula. Limited information is available on the continued use of bevacizumab in patients with other fistulae. In cases of internal fistula not arising in the GI tract, discontinuation of bevacizumab should be considered.

Wound healing complications: Wound healing complications such as wound dehiscence have been reported in patients receiving bevacizumab. In an analysis of pooled data from two trials in metastatic colorectal cancer, patients undergoing surgery 28-60 days before study treatment with 5-FU/LV plus bevacizumab did not appear to have an increased risk of wound healing complications compared to those treated with chemotherapy alone (Scappaticci et al., 2005). Surgery in patients currently receiving bevacizumab is not recommended. No definitive data are available to define a safe interval after bevacizumab exposure with respect to wound healing risk in patients receiving elective surgery; however, the estimated half life of bevacizumab is 21 days. Bevacizumab should be discontinued in patients with severe wound healing complications.

If patients receiving treatment with bevacizumab require elective major surgery, it is recommended that bevacizumab be held for 4-8 weeks prior to the surgical procedure. Patients undergoing a major surgical procedure should not begin or restart bevacizumab until 4 weeks after that procedure (in the case of high-risk procedures such as liver resection, thoracotomy, or neurosurgery, it is recommended that chemotherapy be restarted no earlier than 6 weeks and bevacizumab no earlier than 8 weeks after surgery).

Hemorrhage: Overall, grade 3 and 4 bleeding events were observed in 4.0% of 1132 patients treated with bevacizumab in a pooled database from eight phase I, II, and III clinical trials in multiple tumor types (bevacizumab Investigator Brochure, October 2005). The hemorrhagic events that have been observed in bevacizumab clinical studies were predominantly tumor-associated hemorrhage (see below) and minor mucocutaneous hemorrhage.

Tumor-Associated Hemorrhage: Major or massive pulmonary hemorrhage or hemoptysis has been observed primarily in patients with NSCLC. Life-threatening and fatal hemoptysis was identified as a bevacizumab-related adverse event in NSCLC trials. These events occurred suddenly and presented as major or massive hemoptysis. Among the possible risk factors evaluated (including squamous cell histology, treatment with anti-rheumatic/anti-inflammatory drugs, treatment with anticoagulants, prior radiotherapy, bevacizumab therapy, previous medical history of atherosclerosis, central

tumor location, and cavitation of tumors during therapy), the only variables that showed statistically significant correlations with bleeding were bevacizumab therapy and squamous cell histology.

In Study E4599, in which squamous cell carcinoma was excluded, the rate of any type of Grade ≥ 3 hemorrhage was 1.0% in the control arm (carboplatin and paclitaxel) versus 4.1% in the carboplatin and paclitaxel + bevacizumab arm (Sandler et al. 2006).

GI hemorrhages, including rectal bleeding and melena have been reported in patients with CRC, and have been assessed as tumor-associated hemorrhages.

Tumor-associated hemorrhages were also seen rarely in other tumor types and locations, including a case of CNS bleeding in a patient with hepatoma with occult CNS metastases and a patient who developed continuous oozing of blood from a thigh sarcoma with necrosis.

Mucocutaneous Hemorrhage: Across all bevacizumab clinical trials, mucocutaneous hemorrhage has been seen in 20%-40% of patients treated with bevacizumab. These were most commonly NCI-CTC Grade 1 epistaxis that lasted less than 5 minutes, resolved without medical intervention and did not require any changes in bevacizumab treatment regimen.

There have also been less common events of minor mucocutaneous hemorrhage in other locations, such as gingival bleeding and vaginal bleeding.

Reversible Posterior Leukoencephalopathy Syndrome: There have been rare reports of bevacizumab-treated patients developing signs and symptoms that are consistent with RPLS, a rare neurologic disorder that can present with the following signs and symptoms (among others): seizures, headache, altered mental status, visual disturbance, or cortical blindness, with or without associated hypertension. Brain imaging is mandatory to confirm the diagnosis of RPLS. In patients who develop RPLS, treatment of specific symptoms, including control of hypertension, is recommended along with discontinuation of bevacizumab. The safety of reinitiating bevacizumab therapy in patients previously experiencing RPLS is not known (Glusker et al. 2006; Ozcan et al. 2006).

Congestive Heart Failure: In clinical trials CHF was observed in all cancer indications studied to date, but predominantly in patients with metastatic breast cancer. In the Phase III clinical trial of metastatic breast cancer (AVF2119g), 7 (3%) bevacizumab-treated patients experienced CHF, compared with two (1%) control arm patients. These events varied in severity from asymptomatic declines in left ventricular ejection fraction (LVEF) to symptomatic CHF requiring hospitalization and treatment. All the patients treated with bevacizumab were previously treated with anthracyclines (doxorubicin cumulative dose of 240–360 mg/m^2). Many of these patients also had prior radiotherapy to the left chest wall. Most of these patients showed improved symptoms and/or left ventricular function following appropriate medical therapy (Miller et al. 2005).

In a randomized, Phase III trial of patients with previously untreated metastatic breast cancer (E2100), the incidence of LVEF decrease (defined as NCI-CTC Grade 3 or 4) in the paclitaxel + bevacizumab arm was 0.3% versus 0% for the paclitaxel alone arm.

No information is available on patients with preexisting CHF of New York Heart Association (NYHA) Class II–IV at the time of initiating bevacizumab therapy, as these patients were excluded from clinical trials.

Prior anthracyclines exposure and/or prior radiotherapy to the chest wall may be possible risk factors for the development of CHF. Caution should be exercised before initiating bevacizumab therapy in patients with these risk factors.

A Phase II trial in patients with refractory acute myelogenous leukemia reported 5 cases of cardiac dysfunction (CHF or LVEF decrease to < 40%) among 48 patients treated with sequential cytarabine, mitoxantrone, and bevacizumab. All but 1 of these patients had significant prior exposure to anthracyclines as well (Karp et al. 2004).

Other studies in patients with various tumor types and either a history of anthracycline exposure or concomitant use with bevacizumab are ongoing.

Patients receiving concomitant anthracyclines or with prior exposure to anthracyclines should have a baseline MUGA scans or echocardiograms (ECHOs) with a normal LVEF.

Neutropenia: Increased rates of severe neutropenia, febrile neutropenia, or infection with severe neutropenia (including some fatalities) have been observed in patients treated with some myelotoxic chemotherapy regimens plus bevacizumab in comparison to chemotherapy alone (Sandler et al. 2006).

Additional Adverse Events: See the bevacizumab Investigator Brochure for additional details regarding the safety experience with bevacizumab.

Likely (> 20%)	Less Likely (≤ 20%)	Rare but Serious (< 3%)
• Hypertension • Reproductive system and breast disorders-Other(ovarian failure)[10] • Thrombocytopenia • Lymphopenia	• Anemia • Febrile neutropenia • Supraventricular tachycardia • Vertigo • Abdominal Pain • Colitis • Constipation • Diarrhea • Dyspepsia • Gastrointestinal hemorrhage[2] • Gastrointestinal obstruction[3] • Ileus • Mucositis oral • Nausea • Vomiting • Fatigue • Infusion related reaction • Non-cardiac chest pain • Pain • Allergic reaction • Infection[6]	• Blood and lymphatic system disorders-Other (renal thrombotic microangiopathy) • Acute coronary syndrome • Heart failure • Left ventricular systolic dysfunction • Myocardial infarction • Ventricular arrhythmia • Ventricular fibrillation • Gastrointestinal fistula[1] • Gastrointestinal perforation[4] • Gastrointestinal ulcer[5] • Anaphylaxis • Gastrointestinal anastomotic leak • Intracranial hemorrhage • Ischemia cerebrovascular • Reversible posterior leukoencephalopathy syndrome • Acute kidney injury

	• Infections and infestations-Other (peri-rectal abscess) • Wound dehiscence • Alanine aminotransferase increased • Alkaline phosphatase increased • Aspartate aminotransferase increased • Blood bilirubin increased • Cardiac troponin I increased • Neutrophil count decreased • Weight loss • White blood cell decreased • Anorexia • Arthralgia • Musculoskeletal and connective tissue disorder-Other (bone metaphyseal dysplasia)[7] • Myalgia • Osteonecrosis of jaw[8] • Dizziness • Headache • Peripheral sensory neuropathy[9] • Syncope • Hematuria • Proteinuria • Vaginal hemorrhage • Allergic rhinitis • Cough • Dyspnea • Epistaxis • Hoarseness • Pruritus • Rash maculo-papular • Urticaria • Thromboembolic event	• Renal and urinary disorders-Other (Nephrotic Syndrome) • Urinary fistula • Vaginal fistula • Bronchopleural fistula, Bronchopulmonary hemorrhage • Respiratory thoracic and mediastinal disorders- Other (nasal-septal perforation) • Respiratory thoracic and mediastinal disorders- Other (tracheo-esophageal fistula) • Vascular disorders-Other (arterial thromboembolic event)**[11] • Cellulitis • Posterior reversible encephalopathy syndrome (PRES) • Non-mandibular osteonecrosis • Fetal abnormalities

[1]Gastrointestinal fistula may include: Anal fistula, Colonic fistula, Duodenal fistula, Esophageal fistula, Gastric fistula, Gastrointestinal fistula, Rectal fistula, and other sites under the GASTROINTESTINAL DISORDERS SOC.

[2]Gastrointestinal hemorrhage may include: Colonic hemorrhage, Duodenal hemorrhage, Esophageal hemorrhage, Esophageal varices hemorrhage, Gastric hemorrhage, Hemorrhoidal hemorrhage, Intra-abdominal hemorrhage, Oral hemorrhage, Rectal hemorrhage, and other sites under the GASTROINTESTINAL DISORDERS SOC.

[3]Gastrointestinal obstruction may include: Colonic obstruction, Duodenal obstruction, Esophageal obstruction, Ileal obstruction, Jejunal obstruction, Rectal obstruction, Small intestinal obstruction, and other sites under the GASTROINTESTINAL DISORDERS SOC.

[4]Gastrointestinal perforation may include: Colonic perforation, Duodenal perforation, Esophageal perforation, Gastric perforation, Jejunal perforation, Rectal perforation, Small intestinal perforation, and other sites under the GASTROINTESTINAL DISORDERS SOC.

[5]Gastrointestinal ulcer may include: Duodenal ulcer, Esophageal ulcer, Gastric ulcer, and other sites under the GASTROINTESTINAL DISORDERS SOC.

[6]Infection may include any of the 75 infection sites under the INFECTIONS AND INFESTATIONS SOC.

[7]Metaphyseal dysplasia was observed in young patients who still have active epiphyseal growth plates.

[8]Cases of osteonecrosis of the jaw (ONJ) have been reported in cancer patients in association with bevacizumab treatment, the majority of whom had received prior or concomitant treatment with i.v. bisphosphonates.

[9]Increased rate of peripheral sensory neuropathy has been observed in trials combining bevacizumab and chemotherapy compared to chemotherapy alone.

[10]Ovarian failure, defined as amenorrhea lasting 3 or more months with follicle-stimulating hormone (FSH) elevation (≥30 mIU/mL), was increased in patients receiving adjuvant bevacizumab plus mFOLFOX compared to mFOLFOX alone (34% vs. 2%). After discontinuation of bevacizumab, resumption of menses and an FSH level <30 mIU/mL was demonstrated in 22% (7/32) of these women. Long term effects of bevacizumab exposure on fertility are unknown.

[11]Arterial thromboembolic event includes visceral arterial ischemia, peripheral arterial ischemia, heart attack, and stroke.

**In the AVF2107study [36, 37], there was a 1% incidence of arterial thromboembolic events (which include myocardial ischemia/infarction, transient ischemia attack, cerebrovascular accident/stroke and angina/unstable angina) in the IFL + placebo arm versus 3% in the IFL + bevacizumab arm. A pooled analysis of the rate of arterial TE events from 5 randomized studies showed that treatment with bevacizumab increased the risk of these events two-to-three fold (up to 5%). Furthermore, certain baseline characteristics, specifically age> 65 years and prior arterial TE event, conferred additional risk.

Also reported on Bevacizumab (rhuMAb VEGF) trials but with the relationship to Bevacizumab (rhuMAb VEGF) still undetermined:

- Blood and lymphatic system disorders - Other (idiopathic thrombocytopenia purpura)
- Disseminated intravascular coagulation
- Pericardial effusion
- Gait disturbance
- Sudden death NOS
- Hepatic failure
- Infections and infestations - Other (aseptic meningitis)
- Platelet count decreased
- Hyponatremia
- Musculoskeletal and connective tissue disorder - Other (aseptic necrotic bone)
- Musculoskeletal and connective tissue disorder - Other (myasthenia gravis)
- Dysgeusia

- Peripheral motor neuropathy
- Seizure
- Confusion
- Adult respiratory distress syndrome
- Pneumonitis
- Pneumothorax
- Pulmonary hypertension
- Palmar-plantar erythrodysesthesia syndrome
- Skin ulceration

Note: Bevacizumab (rhuMAb VEGF) in combination with other agents could cause an exacerbation of any adverse event currently known to be caused by the other agent, or the combination may result in events never previously associated with either agent

1.3 Other Study Drug(S) Background

a. Cyclophosphamide And Topotecan

As single agents, cyclophosphamide and topotecan are used in the treatment of a variety of pediatric tumors. The combination of cyclophosphamide and topotecan has been demonstrated to be an active chemotherapeutic regimen for treatment of relapsed/refractory Ewing sarcoma, rhabdomyosarcoma, neuroblastoma, Wilms' tumor, germ cell tumors, and brain tumors.[69] Cyclophosphamide plus topotecan has also shown activity in patients with untreated metastatic neuroblastoma. [25] This activity is attributed to synergistic stabilization of DNA-topoisomerase I adducts by topotecan which are formed after cyclophosphamide-induced DNA damage.

With regard to CNS malignancies, cyclophosphamide is an active chemotherapeutic agent in medulloblastoma and is part of the first line chemotherapy regimen for ependymoma.[26] However, some patients with recurrence will not have had prior exposure to this agent. Patients ≥ 3 years of age with standard risk medulloblastoma are often treated with vincristine, cisplatin and lomustine (without cyclophosphamide), and patients ≥ 3 years of age with completely resected ependymoma will not have received chemotherapy as part of their initial treatment regimen. Topotecan been demonstrated by St. Jude investigators to be active versus medulloblastoma in a phase II window study when administered as a 30 minute infusion daily x 5 days, as will be used in this study.[27] Using a 72-hour infusion schedule of topotecan, Pediatric Oncology Group (POG) investigators reported that 5 of 17 patients with ependymoma achieved stable disease.[28] Children's Cancer Study Group (CCG) investigators performed a phase I study using a 21-day continuous infusion and reported that 2 of 3 patients with ependymoma achieved objective responses.[29]

This study will focus primarily on the evaluation of the efficacy of the combination CTB in a restricted group of pediatric cancer patients, those with diagnoses of Ewing's sarcoma and neuroblastoma. Prior data in patients with Ewing's sarcoma and neuroblastoma have demonstrated efficacy in second line therapy with the combination cyclophosphamide and topotecan, both agents known to demonstrate single agent activity in these diseases. Importantly, objective response data has been published in children with neuroblastoma and Ewing's sarcoma at the time point indicated in the primary study objective of this study, after 2 cycles of therapy, which can be used for comparison, Saylors et al reported response rates after 2 cycles of

cyclophosphamide/topotecan as 4/13 (30.8%) for neuroblastoma patients and 5/17 (29.4%) Ewing's sarcoma patients. Given the similar response rates (~30%) in historical phase II data with the chemotherapy backbone cyclophosphamide and topotecan, it was decided that the disease types neuroblastoma and Ewing's sarcoma would be evaluated in a single cohort .[69] Based upon this information, it is anticipated that the risk of encountering differing response rates in the disease groups will be minimal. Although the response rate that will determine the success of the combination of drugs in this trial will be based upon composite data from both disease groups, subpopulation response rates will be assessed. To safeguard against unequal proportions of patients from each of the disease group being enrolled on this study, the accrual of patients will be stratified.

This study is intended for treatment in patients after first line treatment. Standard frontline chemotherapy for patients with Ewing's sarcoma consists of the agents of vincristine, ifosfamide, doxorubicin, dactinomycin, and etoposide. Standard frontline chemotherapy for patients with neuroblastoma consists of the agents, cyclophosphamide, doxorubicin, vincristine, cisplatin, etoposide in combination with immunotherapy consisting of a monoclonal antibody directed to GD2. Given the limited overlap with first line therapy, it is anticipated that there will be activity as well in this trial.

1.4 Study Rationale

This study will evaluate the safety and efficacy of the chemotherapeutic regimen cyclophosphamide and topotecan in combination with the novel agent bevacizumab (CTB). The schedule that will be used will be the 5-day infusion schedule every 21 days of cyclophosphamide and topotecan. The interest in incorporating bevacizumab with this standard salvage regimen is based upon preclinical data demonstrating activity in pediatric and adult solid tumors. Bevacizumab has been widely demonstrated to inhibit angiogenesis and tumor growth in numerous tumor models including breast, prostate, rhabdomyosarcoma, and Wilms' tumor. Synergism with standard cytotoxic agents has been demonstrated including cyclophosphamide and topotecan. We hypothesize that the addition of bevacizumab to the standard regimen cyclophosphamide and topotecan will show enhanced clinical efficacy without additive toxicity.

In this study, we propose a novel schedule of administration of bevacizumab which may enhance the effect of cytotoxic chemotherapy with cyclophosphamide and topotecan. Bevacizumab will be administered 3 days prior to administration of cytotoxic chemotherapy. The rationale for this approach is summarized as follows. Tumor vascular volume, microvessel density and interstitial fluid pressure are decreased in response to bevacizumab therapy.[30] It has been proposed that blood vessel stabilization may occur given the decrease in interstitial fluid pressure, allowing for improved delivery of chemotherapy to tumor cells. This may explain the synergistic effects of this agent with conventional chemotherapy regimens.[31, 32] In animal models, Tong et al demonstrated that treatment with DC101 targeting anti-VEGF-R2 resulted in pruning of the vasculature, more uniform pericyte coverage of the remaining vessels, and a slight oncotic pressure gradiant facilitating transvascular delivery of macromolecules. [31] R. Jain et al demonstrated that bevacizumab is most effective when given several days prior to conventional chemotherapeutic delivery at a time when tumor blood vessel "normalization" and drug delivery are most optimal.[33] This window of vessel normalization allows for greater influx or convection of chemotherapeutic agents into the tumor which is associated with a decrease in interstitial hypertension and peritumoral

edema and therefore a reduction in the efflux or convection out of the tumor of chemotherapy allowing for greater effect as well as growth factors, and cancer cells form the tumor margins reducing metastatic dissemination. The mechanism of normalization lies in that VEGF overexpression as occurs in most malignancies as discussed above results in highly abnormal vasculature with limited pericyte covering. In fact a precise regulation of the spatial and temporal expression of VEGF is required for normal vasculature. VEGF blockade has been shown to prune immature and leaky vessels with resultant decrease in interstitial hypertension, peri-tumor edema. Recent studies by Dickson et al corroborated these findings. [34] After a single dose of bevacizumab, a progressive decrease in tumor microvessel density in established orthotopic neuroblastoma xenografts was reported to < 30% of control was observed within 7 days. Assessment of the tumor microenvironment revealed a rapid, sustained decrease in both tumor vessel permeability and tumor interstitial fluid pressure, whereas intratumoral perfusion, as assessed by contrast-enhanced ultrasonography, was improved, although this latter change abated by 1 week. Intratumoral drug delivery mirrored these changes. Penetration of chemotherapy was improved by 81% when given 1-3 days after bevacizumab compared with when both drugs were given concomitantly or 7 days apart. Administration of topotecan to tumor-bearing mice 3 days after bevacizumab resulted in greater tumor inhibition (36% of control size) than with monotherapy (88% bevacizumab, 54% topotecan) or concomitant administration of the two drugs (44%). These findings provide the rationale for the novel schedule of administration of bevacizumab proposed in this trial in which bevacizumab will administered 3 days prior to conventional chemotherapy.

1.5 Rationale For Correlative Studies

This study will also include several mandatory exploratory biologic correlative studies to help determine the angiogenic profile of patients at baseline and following treatment with the bevacizumab-containing cytotoxic chemotherapy regimen.

1.5.1 Rationale for Investigating EPCs/HPCs

All tumors require their own blood supply through several means including angiogenesis-cooption, branching from the existing vasculature, and vasculogenesis-recruitment of *de novo* single cells similar to processes observed in embryonic development.

In response to VEGF-A and PlGF (placental growth factor, which signals via VEGFR1 only) EPCs and HPCs proliferate and mobilize from the bone marrow into the circulation for recruitment to the primary tumor.[35, 36] This “angiogenic switch”, supports an extensive vasculature network and has been shown to be associated with a worse prognosis.[37, 38] In particular, $VEGFR1^{+}$ $CD68^{+}$ stromal hemangiocytes within tumor tissue correlated with more aggressive subtypes of Non-Hodgkins lymphoma.[39] Similarly, VEGFR1 and VEGFR2 expression with solid tumors has been associated with more aggressive stage (Ref). Early myeloid HPCs as well as tumor-associated myeloid cells and macrophages (TAMs) have been shown to migrate along blood vessels promoting tumor cell extravasation and invasion into adjacent tissue. These myeloid cell infiltrates have been associated with higher stage tumors and worse prognosis. [36, 38] Moreover, HPCs may have additional roles in local invasion since these cells are commonly present at the peripheral rim of tumors, a site known as the invasive front.[71] Because of their critical contribution to primary tumor growth and invasion, these bone marrow-derived progenitor cells may also participate in metastasis.

At distant sites of future metastasis, bone marrow-derived VEGFR1$^+$ HPCs have been identified prior to evidence of tumor cell spread which establish a conducive microenvironment termed by Lyden et al as the “pre-metastatic niche”. [40] Following the arrival of tumor cells, VEGFR2$^+$ EPCs contribute to new blood vessel formation in micrometastases leading to progression of the metastatic lesions.

Conceivably, HPCs may support tumor cell homing in other niches, such as in the bone marrow microenvironment possibly favoring dormancy or promoting relapse of residual tumor cells. As these bone marrow-derived cells are important in tumor invasion and metastasis, HPCs and EPCs can potentially represent prognostic indicators obtained not only from tissue samples, but also from the blood. The biology of mobilization of HPCs and EPCs during tumorigenesis and metastasis therefore makes enumeration of these cells in circulation ideal surrogate markers to assess response to VEGF-A targeted therapies.

VEGFR2$^+$ circulating EPCs are elevated in the blood of patients in several adult cancers including breast cancer and lung cancer.[41, 42] There have been reports that elevated levels of circulating EPCs and serum VEGF are correlated with poor overall survival in adult Non- Small Cell Lung Cancer. [41] In addition, these markers may have utility in gauging treatment response, as those patients that were poor responders to chemotherapy treatment had persistently elevated circulating EPC levels. [42, 43] Furthermore, recent investigation has shown that the circulating endothelial progenitor marker CD133 can predict colon cancer recurrence. [44] Recently work has been performed in adult patients with non-Hodgkin’s lymphoma that demonstrated elevations in CEPs in patients compared to controls and these levels correlated with treatment response. [45]

There is limited information utilizing VEGFR1$^+$ HPC levels as a biomarker for malignant disease. However, preclinical preliminary data from our laboratory shows that there is elevation in HPCs in pediatric patients with rhabdomyosarcoma and adult patients with breast cancer compared to healthy controls and these levels correlate with more advanced disease and treatment response (Figure 2 & Table 1). Furthermore, recent work by Kosaka et al has demonstrated that a high-risk group of gastric cancer patients can be identified by vascular endothelial growth factor receptor-1 overexpression in the peripheral blood of these patients.[46] Mononuclear cell-derived VEGFR1 mRNA was increased in those patients with advanced clinical stage, who either presented with local invasive disease beyond the muscularis propria or with distant metastases. More importantly and in keeping with our findings that VEGFR1 may be a prognostic marker of metastatic progression, VEGFR1 mRNA was elevated in those patients that developed postoperative recurrence. These findings suggest that this marker may be useful in determining risk of recurrence and may be useful in assessing treatment response by measuring VEGFR1$^+$ HPC levels in pediatric patients with solid tumors receiving treatment with Bevacizumab and other anti-angiogenic agents. We believe that using circulating VEGFR2$^+$ EPCs and VEGFR1$^+$ HPCs in combination will prove to be a powerful biomarker tool and guide risk stratification, treatment decisions and timing and duration of therapy.

Bone marrow-derived HPCs and EPCs play a critical role in the development of *de novo* tumor vasculature as well as in providing a supportive framework for tumor and metastatic progression. HPCs may represent novel biomarkers not only for determining optimal biologic dosing and treatment response but also aid in patient stratification and identify those patients that may benefit most from targeted anti-angiogenic therapies. Determining the presence of these cells and other key molecular markers in the setting of targeted bevacizumab therapy will be studied. These cellular and molecular markers can identify those patients that will benefit most from these anti-angiogenic therapies and the best approach to timing and duration of therapy. Genetic analysis of these cells may also uncover new anti-angiogenic targets as well.

1.5.2 Soluble Markers Of Angiogenesis For The Assessment Of Biological Activity During Bevacizumab Based Therapy

Tumor regression in response to agents such as bevacizumab is usually measured by conventional non-invasive imaging techniques such as computed tomography (CT) and magnetic resonance imaging (MRI). However, because of the cost and the patient inconvenience involved such techniques are not feasible for regular target modulation analysis. In contrast, the measurement of serum and plasma angiogenic biomarkers offers an alternative approach to assess biological responses during treatment. Previous studies have shown that several soluble molecules including VEGF, basic fibroblast growth factor (bFGF) and several endothelial cell-associated molecules provide an effective biomarker profile in response to targeted anti-angiogenic agents. [47-49] This is based on the rationale that the concentration of these angiogenic factors in the plasma may reflect the hypoxic status of a tumor and may increase in response to effective blockade of VEGF receptors.

Abnormalities in other plasma indices of angiogenesis, such as angiopoietins (Ang-1, Ang-2), as well as the soluble receptors Flt-1 (sFlt-1) and Tie 2 (sTie-2) have also been shown to correlate with a tumors response to the blockade of VEGF activity. [50, 51] In addition, tumors that are subjected to therapy have been shown to upregulate a number of other angiogenic growth factors, including placenta growth factor (PlGF), in a tumor-specific pattern. Hence following the expression of PlGF in patients undergoing antiangiogenic treatment provides an avenue to identify treatment effectiveness. [52] Though not comprehensive, we hypothesize that including the measurement of the expression of angiogenic factors (VEGF, bFGF), angiopoietins (Ang-1, Ang-2), angiogenesis modulators (sVEGFR, sTie-2) and angiogenesis enhancers (PlGF) will provide specific and sensitive biomarker profile on the action of bevacizumab and offer important clinical correlative analysis.

1.5.3 Understanding Tumor-Related Processes Through Metabolic Profiling.

Metabolic profiling can be used to differentiate between different cancer cell lines and to monitor metabolic processes in cancer cells during apoptosis. Metabolomics has been used to study the changes in hypoxic regions of tumors that develop when a tumor outgrows its vasculature. [60] In such tumors, over-expression of hypoxia inducible factor (HIF-1) co-exists with increased glucose transporters, glycolytic enzymes and growth factors (such as VEGF). The rate of glycolysis by NMR increases along with induction of angiogenesis. Previous studies with anti-mitotic drugs show the decreased bioenergetic status of the tumor is accompanied by an increase in specific biomarkers. [61] With a novel targeted agent (imatinib for BCR-ABL inhibition, for example), the metabolic signature is

different from classic chemotherapeutic agents, indicating that these methods can be applied to different classes of therapeutics, work now performed in the Gore laboratory.[53-66]

Additional studies will investigate the interaction between bevacizumab, cyclophosphamide and topotecan. The results of the standard pharmacokinetic and pharmacodynamic variables, in conjunction with the parallel MRS metabolic information, will suggest the relative contribution that each individual agent makes within the treatment regimen. Plasma levels of each drug will be determined at defined time points throughout protocol therapy. At defined time-points, MRI scanning will be conducted in conjunction with blood sampling for MR spectroscopy. Tumor and normal tissues /bone marrow can be used for Western blot and gene expression analysis, as is appropriate for normal clinical care of pediatric patients with refractory cancers. For patients who do not require biopsies for their general clinical care, peripheral blood specimens can be evaluated.

These experiments reveal how the combination of bevacizumab, cyclophosphamide and topotecan affects the bioavailability, metabolic and biochemical, and anti-tumor activity of each of the component drugs *in vivo*. MRI/NMR assessment will be of special interest, because at the current time, there are no data regarding non-invasive techniques for measuring metabolic response to treatment in pediatric tumors.

1.5.4 The Impact Of Bevacizumab On Growth And Development In Children.

Reversible skeletal lesions have been reported in the literature in an anecdotal case of a child with cutaneovisceral angiomatosis treated with a short course of bevacizumab.[67] Asymptomatic metaphyseal bone lesions were identified which resolved after discontinuation of the bevacizumab. To date, there have been additional cases reported following treatment with bevacizumab in children and adults; however, follow-up has been limited. In this study, patients will be monitored for changes in the growth plates related to treatment with bevacizumab.

2.0 OBJECTIVES

2.1 Primary

2.1.1 To determine the efficacy of the bevacizumab in combination with cyclophosphamide and topotecan (CTB) in patients with relapsed/refractory Ewing's Sarcoma and neuroblastoma as measured by objective response rate (CR/PR) after 2 cycles of treatment and duration of response.

2.2 Secondary

2.2.1 To evaluate the safety and tolerability of bevacizumab in combination with cyclophosphamide and topotecan.

2.2.2 To determine the efficacy of combining bevacizumab with cyclophosphamide and topotecan by measuring overall survival (OS) and time to progression (TTP).

2.2.3 To determine the pharmacokinetic profile of bevacizumab when combined with cyclophosphamide and topotecan.

2.3 Exploratory

2.3.1 To determine the expression of angiogenesis associated biomarkers in serum in patients with relapsed/refractory Ewing's sarcoma and neuroblastoma treated with CTB.

2.3.2 To determine the angiogenic profile of relapsed/refractory Ewing's sarcoma and neuroblastoma treated with CTB.

2.3.3 To determine the magnetic resonance spectroscopy profile of changes induced by CTB in peripheral blood mononuclear cells and tumor tissues compared to baseline.

2.3.4 To determine the impact of bevacizumab on growth and development in children.

3.0 STUDY DESIGN

3.1 Description of the Study

This is a multi-center, open label phase II study evaluating the safety and efficacy of the novel combination of agents consisting of bevacizumab, cyclophosphamide, and topotecan.

3.2 Rationale for Study Design

3.2.1 The study is designed to determine the efficacy and further evaluate the safety profile of bevacizumab given in combination with cyclophosphamide and topotecan. The schedule of administration of bevacizumab will be unique in that the Bevacizumab will be administered 3 days prior to administration of cytotoxic chemotherapy based upon data by Jain et al demonstrating that bevacizumab is most effective when given several days prior to conventional chemotherapeutic delivery to allow for normalization of tumor blood vessels. The chemotherapy is then administered at a time when tumor blood vessel "normalization" and drug delivery are most optimal. This window of vessel normalization allows for greater influx or convection of chemotherapeutic agents into the tumor which is associated with a decrease in interstitial hypertension and peritumoral edema and therefore a reduction in the efflux or convection out of the tumor of chemotherapy allowing for greater effect as well as growth factors, and cancer cells form the tumor margins reducing metastatic dissemination. It is hoped that this schedule may impact ultimately on efficacy of this combination. This study will also incorporate bevacizumab at the standard dose established in adult and pediatric trials of 15 mg/Kg IV Q 3 weeks that was demonstrated to be safe. In the event of inordinate toxicity, the dose of Bevacizumab will be eliminated. Cyclophosphamide and topotecan will be administered at a fixed dose according to the conventional 5-day schedule of administration.

3.2.2 The tumor types to be evaluated in this study will incorporate tumors for which similar historical efficacy data is available including Ewing's sarcoma and neuroblastoma.

3.3 Outcome Measures

3.3.1 Primary Outcome Measures

The primary efficacy endpoint will be the objective response rate (CR/PR) after 2 cycles of treatment and duration of response according to the Revised RECIST guideline (version 1.1). Patients who experience progression of disease prior to receiving the second cycle of therapy will be considered a treatment failure. If appropriate, patients may be evaluated by other guidelines suitable for their disease (e.g., neuroblastoma). The variables will be the tumor measurements and assessment of non-target lesions by radiographic assessment.

3.3.2 Secondary Outcome Measures

Secondary efficacy endpoints will include time to tumor progression, progression-free survival, and evaluation of pharmacodynamic parameters. Duration of disease stabilization will also be assessed.

3.3.3 Safety Outcome Measures

Safety Assessment Methods
Safety will be assessed by physical examination, interim history, and laboratory assessments. Adverse events will be graded according to the NCI-CTCAE, version 4.0

Safety Laboratory Assessments
The local laboratory of the institution where the trial is being conducted will be used to process the laboratory results. Clinically significant laboratory abnormalities (e.g., those that lead to some sort of intervention) are to be assessed as adverse events and recorded on the appropriate CRF.

Patients will be considered evaluable for response if they have received at least 1 day of protocol therapy. Patients will be considered evaluable for toxicity if they have completed at least one cycle of treatment.

4.0 SAFETY PLAN

See Section 4.1 for complete details of the safety evaluation for this study.

4.1 General Plan To Manage Safety

a. Bevacizumab-Specific

A number of measures will be taken to ensure the safety of patients participating in this trial. These measures will be addressed through exclusion criteria (see Section 5.3) and routine monitoring as follows.

Patients enrolled in this study will be evaluated clinically and with standard laboratory tests before and at regular intervals during their participation in this study. Safety evaluations will consist of medical interviews, recording of adverse events, physical examinations, blood pressure, and laboratory measurements (performed by local laboratories, see Section 8.0). Patients will be evaluated for adverse events (all grades), serious adverse events, and adverse events requiring study drug interruption or discontinuation at each study visit for the duration of their participation in the study.

Patients discontinued from the treatment phase of the study for any reason will be evaluated ~30 days (28–42 days) after the decision to discontinue treatment (see Section 7.1.3).

Specific monitoring procedures are as follows:

1. Hypertension: Hypertension will be monitored through routine evaluation of blood pressure prior to each bevacizumab treatment. Optimal control of blood pressure according to standard public health guidelines is recommended for patients on treatment with or without bevacizumab. Patients who develop hypertension while on study, as defined by systolic BP ≥140 and/or diastolic BP ≥ 90 mmHg, or greater than 20% above upper limit of normal (ULN) for age, should be treated with appropriate medical therapy, initially with a single agent antihypertensive medication. A second antihypertensive agent should be added if suboptimal control continues. For controlled hypertension (sBP <140 and/or dBP < 90 mmHg or less than 20% above ULN for age), patients may continue study treatment without interruption. For persistent or symptomatic hypertension, defined as ≥ grade 2, despite optimal medication, bevacizumab must be held until hypertension is ≤ grade 1. Patients with grade 4 hypertension (hypertensive crisis) or hypertension that persists despite optimal medical management will be discontinued from study treatment. Refer to Appendix G for normal values for blood pressure in children.

2. Proteinuria: Proteinuria will be monitored by urine protein: creatinine (UPC) ratio or dipstick at screening, Day 0 of therapy and every 2 cycles before the next dose of Bevacizumab. For patients with ≥ 2+ protein by dipstick (for younger patients refer to CTCAE V4.0 for values) on Day 0 and subsequent testing after initiation of study therapy, bevacizumab therapy will be held and a 24-hour urine collection will be performed for total protein analysis. If the 24-hour urine protein is < 2 g, then the patient may continue bevacizumab therapy at the same dose without interruption. If the urine protein is 2-3 g/24 hours, bevacizumab therapy will be held for one week, and a 24-hour urine collection will be repeated. Treatment with bevacizumab may be reinstituted after a one or two week delay if the 24 hour protein is < 2 g/24 hours. In instances in which the bevacizumab is held 1-2 weeks, all protocol therapy will be held to ensure that the bevacizumab is given prior to chemotherapy in order to test the hypothesis of this study. Patients will be discontinued from the study therapy if the 24-hour protein is > 3 g/24 hours, if there is a second 24 hour urine protein > 2 g, or if the 24-hour urine protein does not decrease to < 2 g/24 hours after a 2 week delay of dosing. Patients will be discontinued from the study therapy if the 24-hour protein is > 3 g/24 hours, if there is a second 24 hour urine protein > 2 g, or if the 24-hour urine protein does not decrease to < 2 g/24 hours after a 2 week delay of dosing.

3. Elective major surgery: If patients on treatment with bevacizumab require elective major surgery, it is recommended that bevacizumab be held for 4-8 weeks prior to the surgical procedure. Patients undergoing a major surgical procedure should not begin/restart bevacizumab until 4 weeks after that procedure (in the case of high risk procedures such as liver resection, thoracotomy, or neurosurgery, it is recommended that treatment with the chemotherapy combination, CTB should be restarted no earlier 8 wk after surgery).

b. Other Study Drug(s)-Specific

Please see Section 7.1.3 for detailed instructions for the management of study drug–related toxicities.

5.0 STUDY SUBJECTS

5.1 Subject Selection

Investigators will be expected to maintain a screening log of all potential study candidates that includes limited information about the potential candidate (ie. age, sex, date) and the outcome of the screening process (ie. enrolled into study, reason for ineligibility, or refusal to participate).

Screening Failures:
Patients who fail to meet the inclusion and/or exclusion criteria are defined as screen failures. For all screen failures, the investigator will document the patient initials and reason(s) for screen failure in the screening log. A copy of the log should be retained in the investigator's study files.

5.2 Inclusion Criteria

Patients will be included in the study based on the following criteria:

5.2.1 Patients must have histologically confirmed relapsed/refractory Ewing's sarcoma or neuroblastoma.

5.2.2 Patients must have measurable disease (Refer to Section 8.6.1.1 for the definition of measurable disease.) Patients with a diagnosis of neuroblastoma with MIBG avid disease only are permitted to enroll on this study.

5.2.3 Patients must be ≤ 21 years of age at time of diagnosis

5.2.4 Life expectancy ≥ 3 months

5.2.5 Lansky or Karnofsky performance ≥ 70%

5.2.6 Written informed consent

5.2.7 Organ and marrow function defined as follows:

Hematologic function, as follows

- Absolute neutrophil count ≥ 1000/μL
- Platelets ≥ 100 x 10^9/L (without transfusion < 14 days before enrollment)
- Hemoglobin ≥ 9 gm/dl

Renal function, as follows:

- Serum creatinine ≤ ULN for age. Refer to Appendix H for normal values for serum creatinine in children.

- If serum creatinine above these values, the calculated creatinine clearance or radioisotope GFR must be ≥ 60 ml/min/1.73 m^2
- Urinary protein < 2+ (unless total quantitative protein is < 500 mg protein/day as determined by 24 H urine collection). for pediatric patients please refer to the CTCAE V4.0 for values.

  Hepatic function, as follows:

  - Total bilirubin ≤ 1.5x ULN
  - AST and ALT ≤ 2.5x ULN for institution or ≤ 5x ULN for institution if clearly attributable to liver metastases
  - Albumin ≥ 2.5 g/dl.

  Coagulation: INR ≤ 1.5x ULN.

5.2.8 Prior Treatment: Patients must have recovered from the acute toxic effects of all prior chemotherapy, immunotherapy, or radiation therapy prior to entry on study. Patients must have had at least one prior treatment regimen. Patients may have received treatment previously with cyclophosphamide, topotecan, or bevacizumab.

Myelosuppressive chemotherapy: Two weeks must have elapsed since administration of previous chemotherapy.

Biologic agents: At least 2 weeks must have elapsed since the completion of therapy with a monoclonal antibody. Seven days must have elapsed since the last dose of retinoids.

Radiation therapy: For all patients, ≥ 4 weeks must have elapsed for local XRT; ≥ 6 months must have elapsed if prior radiation to ≥ 50% of the pelvis or if substantial bone marrow irradiation. Patients with a history of prior radiation with field including the heart (e.g. mantle) will be excluded.

Stem cell transplant: Patients who have undergone prior stem cell transplantation will not be excluded from study entry. At least 3 months must have elapsed since autologous or allogeneic stem cell transplantation. Patients must have no evidence of active graft versus host disease.

5.3 Exclusion Criteria

a. Disease-Specific Exclusions

5.3.1 Disease-Specific Exclusions

- Patients with centrally-located pulmonary or mediastinal primary tumors or metastases adjacent to or invading large blood vessels.
- Prior left chest wall irradiation or a cumulative anthracycline dose of greater or equal to 300 mg/m^2, unless the ejection fraction or fraction shortening is within normal institutional limits, in which case the patient can be enrolled.

b. General Medical Exclusions

Subjects meeting any of the following criteria are **ineligible** for study entry:

- Inability to comply with study and/or follow-up procedures
- Life expectancy of less than 3 months
- Current, recent (within 4 weeks of the first infusion of this study), or planned participation in an experimental drug study other than a Genentech-sponsored bevacizumab cancer study
- Active second malignancy, other than superficial basal cell and superficial squamous (skin) cell, or carcinoma in situ of the cervix within last five years
- History of other malignancies, except for other solid tumors curatively treated with no evidence of disease for > 3 years prior to enrollment.
- Known infection with human immunodeficiency virus (HIV).

c. Bevacizumab-Specific Exclusions

- Uncontrolled hypertension (sBP >150 mmHg and/or diastol*i*c BP > 100 mmHg, found on two consecutive measurements separated by a one week period of time despite adequate medical support).
- Prior history of hypertensive crisis or hypertensive encephalopathy.
- New York Heart Association (NYHA) Grade II or greater congestive heart failure (see Appendix E).
- History of myocardial infarction or unstable angina within 6 months prior to Day -3.
- History of stroke or transient ischemic attack within 6 months prior to Day -3.
- Known CNS disease, except for treated brain metastases.

  Treated brain metastases are defined as having no evidence of progression or hemorrhage after treatment and no ongoing requirement for dexamethasone, as ascertained by clinical examination and brain imaging (MRI or CT) during the screening period. Anticonvulsants (stable dose) are allowed. Treatment for brain metastases may include whole brain radiotherapy (WBRT), radiosurgery (RS; Gamma Knife, LINAC, or equivalent) or a combination as deemed appropriate by the treating physician. Patients with CNS metastases treated by neurosurgical resection or brain biopsy performed within 3 months prior to Day -3 will be excluded.
- Significant vascular disease (e.g., aortic aneurysm, requiring surgical repair or recent peripheral arterial thrombosis) within 6 months prior to Day -3.
- History of hemoptysis (≥ 1/2 teaspoon of bright red blood per episode) within 1 month prior to Day -3.
- Evidence of bleeding diathesis or significant coagulopathy (in the absence of therapeutic anticoagulation).

- Major surgical procedure, open biopsy, or significant traumatic injury within 28 days prior to Day -3 or anticipation of need for major surgical procedure during the course of the study. (A major procedure constitutes an invasive procedure which requires general anesthetic support, hospitalization, and supportive care such as laparotomy, laminectomy, etc.)
- Core biopsy or other minor surgical procedure, excluding placement of a vascular access device, within 7 days prior to Day 1. (Minor surgical procedures include minimally invasive procedures such as fine needle aspiration, core biopsy, etc requiring little if any supportive care excluding lumbar puncture and bone marrow aspiration/biopsy.)
- History of abdominal fistula or gastrointestinal perforation within 6 months prior to Day -3.
- Serious, non-healing wound, active ulcer, or untreated bone fracture.
- Thrombolytics or treatment doses of warfarin within 28 days of initiating treatment. Patients who require low dose warfarin for central venous catheter patency are allowed to enter if their dose is < 2 mg per day total AND their International Normalized Ratio (INR) is ≤ 1.5.
- Patients requiring treatment doses of heparin for any reason. The use of heparin flushes for maintenance of central venous catheters is permitted.
- Patients requiring aspirin > 325 mg per day or non-steroidal anti-inflammatory medications known to inhibit platelet function. Patients taking cyclooxygenase-2 inhibitors (COX-2) inhibitors are allowed to enroll.
- History or clinical evidence of deep venous thrombosis including pulmonary embolus within 6 months of treatment.
- Patients with proteinuria > 1+ on urine dipstick or UPC ratio ≥ 1.0 at screening. If >1+ proteinuria is detected on surveillance, a 24-hour collection must be performed if eligibility is desired. Patients with a 24-hour urine protein content of ≤ 500 mg are eligible.
- Known hypersensitivity to any component of bevacizumab.
- Pregnancy (positive pregnancy test) or lactation. (An effective means of contraception (men and women) in subjects of child-bearing potential must be used.)

6.0 STUDY DESIGN

6.1 Treatment Plan

The treatment schedule for this study will consist of a 21-day cycle. Dose modification will only occur with administration of the investigational agent, bevacizumab.

The schedule of administration is summarized as follows. Administration of bevacizumab will precede the administration of the cyclophosphamide and topotecan by 3 days (Day -3) to allow for vascular stabilization prior to initiation of chemotherapy. Refer to Section 7.1.1 for administration guidelines for bevacizumab.

The chemotherapy backbone will consist of cyclophosphamide and topotecan administered as follows: cyclophosphamide 250 mg/m^2/day IV over 30 minutes ± 5 minutes on Days 0-4 followed by topotecan 0.75 mg/m^2/day IV over 30 minutes ± 5 minutes on Day 0-4 of every cycle. The dosing of cyclophosphamide and topotecan will be fixed.

Dose Escalation Schema

Dose Level	Cyclophosphamide (mg/m2) daily x 5 Q 21 days Days 0-4	Topotecan (mg/m2) daily x 5 Q 21 days Days 0-4	Bevacizumab (mg/kg) Q 3 weeks Day -3
-1	**250 mg**	**0.75 mg**	**-**
1	**250 mg**	**0.75 mg**	**15 mg**

Administration of Filgrastim will begin 24 hours after completion of chemotherapy (Day 5) at a dose of 5 mcg/kg/dose subcutaneously until the ANC is ≥ 1,000/µL for 3 consecutive days or ≥ 10,000/µL for 1 day. Administration of Neulasta is permitted.

Patients can begin subsequent cycles of CTB once the following criteria have been met: 1) after a minimum of 21 days since the start of the previous treatment cycle, 2) ANC ≥ 1000/µL 48 hours after discontinuation of Filgrastim, and 3) platelet count ≥ 100,000/µL.

In the event of development of the following toxicities defined as follows, treatment with bevacizumab will be withdrawn and therapy resumed with cyclophosphamide and topotecan alone:

- If attribution of the event to bevacizumab is felt to be likely or definite
- For any severe adverse event as outlined in Section 7.1.3, Table 1

There will be no intra-patient dose escalation of bevacizumab.

Response will be evaluated by Revised RECIST guideline (version 1.1) after every 2 cycles of therapy (6 weeks). If the response is stable or better, the patient will continue on therapy up to a maximum duration of 1 year as long as their cancer continues to demonstrate response to treatment.

Exploratory biologic correlative studies will be performed during the first and second cycle of treatment. See Section 8.6.

6.2 Number of Centers

This trial will be conducted in the Pediatric Oncology Experimental Therapeutics Investigators' Consortium (POETIC). Approximately 10 centers in the United States and Canada will participate in this study.

6.3 Number of Subjects

The maximum sample size for this study is anticipated to be a total of 29 patients.

6.4 Estimated Study Duration

The accrual period is estimated to be approximately 1 ½ years. The maximum duration of the study will be approximately 2 years.

7.0 STUDY MEDICATION

7.1 Bevacizumab Dosage and Formulation

Bevacizumab is a clear to slightly opalescent, colorless to pale brown, sterile liquid concentrate for solution for intravenous (IV) infusion. Bevacizumab may be supplied in 20-mL (400 mg) glass vials containing 16 mL bevacizumab, respectively (25 mg/mL). Vials contain bevacizumab with phosphate, trehalose, polysorbate 20, and Sterile Water for Injection (SWFI), USP. Vials contain no preservative and are suitable for single use only.

For further details and molecule characterization, see the bevacizumab Investigator Brochure.

7.1.1 Bevacizumab Administration

Bevacizumab will be diluted in a total volume of 100 mL of 0.9% Sodium Chloride Injection, USP. Administration will be as a continuous IV infusion. Anaphylaxis precautions should be observed during study drug administration. It is not necessary to correct dosing based on ideal weight.

The initial dose will be delivered over 90 ± 15 minutes. If the first infusion is tolerated without infusion-associated adverse events (fever and/or chills), the second infusion may be delivered over 60 ± 10 minutes. If the 60-minute infusion is well tolerated, all subsequent infusions may be delivered over 30 ± 10 minutes.

If a subject experiences an infusion-associated adverse event, he or she may be premedicated for the next study drug infusion; however, the infusion time may not be decreased for the subsequent infusion. If the next infusion is well tolerated with premedication, the subsequent infusion time may then be decreased by 30±10 minutes as long as the subject continues to be premedicated. If a subject experiences an infusion-associated adverse event with the 60-minute infusion, all subsequent doses should be given over 90 ± 15 minutes. Similarly, if a subject experiences an infusion-associated adverse event with the 30-minute infusion, all subsequent doses should be given over 60 ± 10 minutes.

7.1.2 Bevacizumab Storage

Upon receipt of the study drug, vials are to be refrigerated at 2°C-8°C (36°F-46°F) and should remain refrigerated until just prior to use. **DO NOT FREEZE. DO NOT SHAKE.** Vials should be protected from light.

Opened vials must be used within 8 hours. **VIALS ARE FOR SINGLE USE ONLY**. Vials used for 1 subject may not be used for any other subject. Once study drug has been added to a bag of sterile saline, the solution must be administered within 8 hours.

7.1.3 Bevacizumab Dose and Toxicity Management

There are no reductions in the bevacizumab dose. If adverse events occur that require holding bevacizumab, the dose will remain the same once treatment resumes. Any toxicities associated or possibly associated with bevacizumab treatment should be managed according to standard medical practice. Discontinuation of bevacizumab will have no immediate therapeutic effect. Bevacizumab has a terminal half-life of 21 days; therefore, its discontinuation results in slow elimination over several months. There is no available antidote for bevacizumab.

Subjects should be assessed clinically for toxicity prior to, during, and after each infusion. If unmanageable toxicity occurs because of bevacizumab at any time during the study, treatment with bevacizumab should be discontinued.

Infusion Reaction: Infusion of bevacizumab should be interrupted for subjects who develop dyspnea or clinically significant hypotension. Subjects who experience a NCI CTCAE v. 4.0 Grade 3 or 4 allergic reaction/hypersensitivity, adult respiratory distress syndrome, or bronchospasm (regardless of grade) will be discontinued from bevacizumab treatment.

The infusion should be slowed to 50% or less or interrupted for subjects who experience any infusion-associated symptoms not specified above. When the subject's symptoms have completely resolved, the infusion may be continued at no more than 50% of the rate prior to the reaction and increased in 50% increments every 30 minutes if well tolerated. Infusions may be restarted at the full rate during the next cycle.

Adverse events requiring delays or permanent discontinuation of bevacizumab are listed below in Table 1.

Regardless of the reason for holding study drug treatment, the maximum allowable length of treatment interruption is 2 months.

Table1

Bevacizumab Dose Management Due to Adverse Events

Event	Action to be Taken
Hypertension	
No dose modifications for grade 1/2 events	
Grade 3	If not controlled to 150/100 mmHg with medication, discontinue bevacizumab.
Grade 4 (including hypertensive encephalopathy)	Discontinue bevacizumab.
Hemorrhage	
No dose modifications for grade 1/2 non-pulmonary and non-CNS events	

Grade 3 Non-pulmonary and non-CNS hemorrhage	Subjects who are also receiving full-dose anticoagulation will be discontinued from receiving bevacizumab. All other subjects will have bevacizumab held until all of the following criteria are met: • The bleeding has resolved and hemoglobin is stable. • There is no bleeding diathesis that would increase the risk of therapy. • There is no anatomic or pathologic condition that significantly increases the risk of hemorrhage recurrence. Subjects who experience a repeat Grade 3 hemorrhagic event will be discontinued from receiving bevacizumab.
Grade 4 non-pulmonary or non-CNS hemorrhage	Discontinue bevacizumab.
Grade 1 pulmonary or CNS hemorrhage	Subjects who are also receiving full-dose anticoagulation will be discontinued from receiving bevacizumab. All other subjects will have bevacizumab held until all of the following criteria are met: • The bleeding has resolved and hemoglobin is stable. • There is no bleeding diathesis that would increase the risk of therapy. • There is no anatomic or pathologic condition that significantly increases the risk of hemorrhage recurrence.
Grade 2, 3, or 4 pulmonary or CNS hemorrhage	Discontinue bevacizumab.
Venous Thrombosis No dose modifications for grade 1/2 events	

Table 1

Bevacizumab Dose Management due to Adverse Events (continued)

Grade 3 or 4	Hold study drug treatment. If the planned duration of full-dose anticoagulation is <2 weeks, bevacizumab should be held until the full-dose anticoagulation period is over. If the planned duration of full-dose anticoagulation is >2 weeks, bevacizumab may be resumed during the period of full-dose anticoagulation if all of the following criteria are met: • The subject must have an in-range INR (usually between 2 and 3) if on warfarin; LMWH, warfarin, or other anticoagulant dosing must be stable prior to restarting bevacizumab treatment. • The subject must not have had a Grade 3 or 4 hemorrhagic event while on anticoagulation.

Arterial Thromboembolic event (New onset, worsening, or unstable angina, myocardial infarction, transient ischemic attack, cerebrovascular accident, and any other arterial thromboembolic event)	
Any grade	Discontinue bevacizumab.
Congestive Heart Failure (Left ventricular systolic dysfunction) No dose modifications for grade 1/2 events	
Grade 3	Hold bevacizumab until resolution to Grade ≤ 1.
Grade 4	Discontinue bevacizumab.
Proteinuria No dose modifications for grade 1/2 events	
Grade 3 (UPC> 3.5, urine collection > 3.5 g/24 hr)	Hold bevacizumab treatment until ≤ Grade 2, as determined by either UPC ratio ≤ 3.5 or 24 hr collection ≤ 3.5 g
Grade 4 (nephritic syndrome)	Discontinue bevacizumab.
GI Perforation	Discontinue bevacizumab.
Fistula	
Any grade (TE fistula)	Discontinue bevacizumab.
Grade 4 fistula	Discontinue bevacizumab.
Bowel Obstruction	
Grade 1	Continue patient on study for partial obstruction NOT requiring medical intervention.

Table 1

Bevacizumab Dose Management due to Adverse Events (continued)

Grade 2	Hold bevacizumab for partial obstruction requiring medical intervention. Patient may restart upon complete resolution.
Grade 3/4	Hold bevacizumab for complete obstruction. If surgery is necessary, patient may restart bevacizumab after full recovery from surgery, and at investigator's discretion.
Wound dehiscence Any grade (requiring medical or surgical therapy)	**Discontinue bevacizumab.**
Reversible Posterior Leukoencephalopathy	
Any grade (confirmed by MRI)	Discontinue bevacizumab.

Other Unspecified Bevacizumab-Related Adverse Events	
Grade 3	Hold bevacizumab until recovery to ≤ Grade 1
Grade 4	Discontinue bevacizumab.

7.1.4 Investigational Agent Management

FDA regulations require investigators to establish a record of the receipt, use, and disposition of all investigational agents. The investigators in this study have the responsibility to assure the FDA that systems for agent accountability are being maintained by investigators in the clinical trials network. Investigators may delegate responsibility for agent ordering, storage, accountability and preparation to a designee in their institutions. However, the investigator is ultimately responsible for all agents shipped in his/her name. The intent of agent accountability is to assure that supplied agents are only used for patients enrolled on this trial. Investigational agents will not be shipped to a site unless all site and study specific regulatory documents have been received by the POETIC Data and Coordinating Center (DCC). The POETIC DCC will notify the site of their activation and provide clearance to order study drug from Fisher Clinical Sciences.

Requests and Shipping

Upon site activation by the POETIC DCC, the site will complete a Drug Shipment Authorization form and submit the document to the Fisher Clinical Sciences at the address listed below:

Fisher Clinical Sciences
Distribution Study Administration
7554 Schantz Road
Allentown, PA 18106
Phone: 610-871-8300
Fax: 610-871-8590
E-mail: distribution.allentown@Thermofisher.com

The information needed for completion Drug Shipment Authorization form is the POETIC protocol number, address, product information and quantity. The POETIC protocol, **POE10-01**, is included on the form. Investigational agents will only be shipped to the investigator's designated shipping address. All changes to the shipping address must be in writing and signed by the investigator or designee.

Requests for investigational agents for new subjects should be submitted the day of registration and no less than 3 business days prior to the start of treatment. Requests for subsequent cycles should be made a minimum of 1 week prior to treatment. Telephone requests will not be accepted.

Investigational agents will only be shipped to the investigator's designated shipping address. All changes to the shipping address must be in writing and signed by the investigator or designee.

Upon receipt of the drug supply, the site must verify and document that correct agent(s), lot numbers, and quantity were received in adequate shipping conditions. Once verified the Packing Slip must be completed, signed and returned to Fisher Clinical Sciences to the following contact and FAX: AOR Administrator, FAX# 610-871-0775. If there are problems with the shipment, the Site must notify the AOR Administrator immediately.

If there are any additional problems contact:

Gregg J. Rieker
Distribution Project Manager
Thermo Fisher Scientific (Fisher Clinical Sciences)
700 Nestle Way
Breinigsville, PA 18031
Phone: 484-538-2139
Fax: 610-871-0711
gregg.rieker@thermofisher.com

7.1.5 Drug Accountability

The study pharmacist at the site will be responsible for handling study drug, preparation of the appropriate dose to be administered, and completion of associated documentary paperwork. Documentation (receipt, transfer, dispensing, or return) will be maintained on the NCI Investigational Drug Accountability Record (DAR). Alternative accountability records may be provided to meet study specific requirements (example – oral medication involving return of meds by patient) beyond the DAR form's capability. A copy of the appropriate accountability record will be sent with the investigational agent.

A DAR must be maintained at each location an agent is stored (example – main pharmacy, satellite, etc). A separate DAR will be maintained for each protocol. Protocols using more than one supplied agent or more than one strength or formulation of the same agent, each agent, strength, and formulation will be stored separately and a separate DAR maintained. Proper completion of the DAR is mandatory, failure to complete all fields as required may prevent future shipments until it is determined appropriate accountability can be maintained. Each time a dose is prepared for a patient, the following information must be recorded: the patient's initials, the patient's study number, the total dose prepared, the number of vials of drug product and diluent used, the number of the lot from which the dose was prepared, and the initials of the person preparing the dose. Audit of the DAR will be conducted at the end of the study and more often if needed. All investigational agents will be stored as per manufacturer recommendations in a secure location away from commercial drug stock that is only accessible to authorized personnel. Each agent is to be stored and accounted for separately by protocol.

Investigational agents requiring storage in refrigerator or freezer must be stored within the temperature range required by the manufacturer upon receipt. The agents must be stored separately by protocol within the refrigerator/freezer. No food products may be stored within the refrigerator/freezer. A thermometer or monitoring device must be utilized to monitor and record temperatures on a log posted on the equipment at a minimum of once daily. Out of range temperatures must be documented with reason for situation and actions taken to correct.

Investigational agents must not be prepared until the subject is present, required testing completed, and the treating physician's orders are received. The investigator will administer the agent only to subjects registered to the study and under the investigator's personal supervision or under the supervision of a sub-investigator responsible to the investigator**.**

In addition to maintaining records of drug dispensation, an appropriate drug preparation log should also be maintained to document how the study drug solution was prepared. Calculations performed for preparation of the drug should be clearly recorded. If the site does not have such logs, Genentech will provide them. These logs are to be maintained by the study pharmacist in the pharmacy throughout the duration of the study and will be periodically verified by a representative of the Sponsor.

7.1.6 Disposition of Used Supplies

All used vials of study drug and diluent must be destroyed in an appropriate manner according to the standard practice at each study center. Destruction of such supplies will be documented, and a representative of the Sponsor will verify disposition records. No other utilization of bevacizumab study drug solution intended for use in this study is authorized by the Sponsor. The Principal Investigator or his/her designee will be responsible for the appropriate handling and disposition of residual study drug.

7.1.7 Inventory of Unused Supplies

Transfer of investigational agents between sites is prohibited unless approved by the DCC and the pharmaceutical management for drug distribution prior to the transfer.

Investigational agents are not interchangeable with approved commercial agents. Correct stock must be used for all doses, if investigational agent is not available for a subject at the scheduled time the subject's appointment must be changed.

Every effort should be made to minimize the amount of agent ordered and returned unused. Unused agents will either be returned or destroyed on-site as directed by the study staff when the study is completed or discontinued; agent is outdated, damaged or unfit for use. Opened or partially used vials/bottles are not to be returned unless specifically requested otherwise in the protocol. Broken vials are to be destroyed at the site, not returned.

If return of investigational agent is not required, the agent will be treated as chemotherapy or biological hazardous waste as appropriate and disposed of in accordance to the policies for hazardous waste management at the Site.

For agents destroyed on-site, the DAR must be completed and faxed to the POETIC DCC. If the investigational agent is returned, the completed DAR must accompany the agent.

Sites must maintain copies of all Requests for Drug Shipment, Packing Slips, and DARs on site, available for monitoring and auditing until final closure of the study. After final closure of the study, forms may be stored off site as per the policy of the site.

7.2 Protocol Specified Chemotherapy

7.2.1 Cyclophosphamide (Cytoxan) NSC #26271

7.2.1.1 Source and Pharmacology:

Cyclophosphamide is an alkylating agent related to nitrogen mustard. Cyclophosphamide is inactive until it is metabolized by P-450 isoenzymes (CYP2B6, CYP2C9 and CYP3A4) in the liver to active compounds. The initial product is 4-hydroxycyclophosphamide (4-HC) which is in equilibrium with aldophosphamide which spontaneously releases acrolein to produce phosphoramide mustard. Phosphoramide mustard, which is an active bifunctional alkylating species, is 10 times more potent in vitro than is 4-HC and has been shown to produce interstrand DNA cross-linking analogous to those produced by mechlorethamine. Approximately 70% of a dose of cyclophosphamide is excreted in the urine as the inactive carboxyphosphamide and 5-25% as unchanged drug. Cyclophosphamide is well absorbed orally with a bioavailability greater than 75%. The plasma half-life ranges from 4.1-16 hours after IV administration and 1.3-6.8 hours after oral administration.

7.2.1.2 Formulation:

Cyclophosphamide for Injection is available as powder for injection or lyophilized powder for injection in 500 mg, 1 gm and 2 gm vials. The powder for injection contains 82 mg sodium bicarbonate/100 mg cyclophosphamide and the lyophilized powder for injection contains 75 mg mannitol/100 mg cyclophosphamide.

7.2.1.3 Storage:

Storage at or below 25ºC (77ºF) is recommended. The product will withstand brief exposures to temperatures up to 30º C (86ºF).

7.2.1.4 Reconstitution:

Cyclophosphamide for Injection: Reconstitute with sterile water or Bacteriostatic water for injection (paraben preserved only) to a concentration of 20 mg/ml. If administered as undiluted drug at the 20 mg/ml concentration, reconstitute with NS only to avoid a hypotonic solution. Cyclophosphamide may be further diluted in dextrose or saline containing solutions for IV use.

7.2.1.5 Stability:

Reconstituted solution with preservative is stable for 24 hours at room temperature, or 6 days if refrigerated. Solution reconstituted without preservative should be discarded after 6 hours.

7.2.1.6 Administration:

In this study, cyclophosphamide will be administered intravenously over a 30 minute period ± 5 minutes on 5 consecutive days.

7.2.1.7 Supplier:

Commercially available from various manufacturers. See package insert for further information

7.2.1.8 Safety Profile:

Likely	Less Likely	Rare but Serious

• Loss of appetite
• Nausea
• Vomiting
• Fewer white blood cells in the blood.
  ○ A low number of white blood cells may make it easier to get infections.
• Hair loss
• Decreased ability of the body to fight infection
• Absence or decrease in the number of sperm which may be temporary or permanent which may decrease the ability to have children
	• Abnormal hormone function which may lower the level of salt in the blood
	• Abdominal pain
	• Diarrhea
	• Fewer red blood cells and platelets in the blood
	  ○ A low number of red blood cells may make you feel tired and weak.
	  ○ A low number of platelets may cause you to bruise and bleed more easily.
	• Bleeding and inflammation of the urinary bladder
	• Absence or decrease monthly periods which may be temporary or permanent and which may decrease the ability to have children
	• Temporary blurred vision
	• Nasal stuffiness at time of IV infusions
	• Skin rash
	• Darkening of areas of the skin and finger nails
	• Slow healing of wounds
	• Infections
	• Metallic taste in mouth
		• Heart muscle damage which may occur with very high doses and which may be fatal
		• Abnormal heart rhythms
		• Damage and scarring of lung tissue which may make you short of breath
		• A new cancer or leukemia resulting from this treatment.
		• Damage or scarring of urinary bladder tissue
		• Severe allergic reaction which can be life threatening with shortness of breath, low blood pressure, rapid heart rate chills and fever
		• Infertility which is the inability to have children

7.2.2 Topotecan, NSC #609699, IND #34494

7.2.2.1 Source and Pharmacology:

Topotecan is a semisynthetic analogue of camptothecin, an alkaloid derived from the camptothecin tree which grows widely throughout Asia. The drug is a specific

Topoisomerase-I inhibitor. Topotecan interferes with the repair activity of topoisomerase I by stabilizing the formation of a covalently bonded DNA-Topoisomerase I complex. Thus, the 5'-phosphoryl terminus of the enzyme catalyzed single strand DNA break remains bound to the tyrosine of the enzyme thereby interfering with replication. The drug exists as two species in equilibrium in aqueous solutions. One is a more active closed-ring lactone and the other a less active open ring form. Acidic conditions favor the closed ring lactone form while basic and physiologic pH favors the open ring form. In human plasma the lactone form is about 21% bound to plasma protein. The drug is excreted 39% in urine and the remainder in the stool, the latter is presumably via biliary excretion. In rats, over 90% of the radioactivity from [^{14}C]topotecan was recovered in stool and urine in the first 96 hours. The half life for the lactone form is 180 minutes.

7.2.2.2 Formulation:

Topotecan is supplied as a lyophilized, light yellow powder in vials containing 4 mg Topotecan AS (as the base) and 48 mg mannitol and 20 mg tartaric acid, NF. The pH is adjusted to 3.0. It has a reverse magenta label for identification purposes.

7.2.2.3 Storage:

Unreconstituted vials are stored at room temperature, 15º-30ºC (59º-86ºF).

7.2.2.4 Reconstitution:

The contents of each 4 mg vial will be reconstituted with 4 mL Sterile Water for Injection, USP, yielding a 1 mg/mL solution of Topotecan AS. The vial can also be reconstituted with Bacteriostatic Water for Injection, USP.

7.2.2.5 Stability:

The vials reconstituted with Sterile Water for Injection, USP, contain no antibacterial preservative and must be used within 8 hours. Those reconstituted with Bacteriostatic Water for Injection, USP, are stable for 21 days when stored in the refrigerator (2-8ºC).

The reconstituted solution will be further diluted to concentrations of 10 mcg/mL to 500 mcg/mL in Dextrose 5% in Water, Normal Saline, or Bacteriostatic Water for Injection, USP. Solutions for infusion in glass or plastic bags are stable at room temperature for 24 hours if reconstituted with Dextrose 5% in Water or Normal Saline and are stable for 7 days if reconstituted with Bacteriostatic Water for Injection, USP. No incompatibilities with other drugs have been described. The drug is stable in IV solutions as stated above.

7.2.2.6 Administration:

In this study, topotecan will be administered intravenously over a 30-minute +/- 5 minutes on five consecutive days. The appropriate amount of drug should be diluted in D_5W yielding a final volume of 25 mL.

7.2.2.7 Supplier:

Commercially available from various manufacturers. See package insert for further information.

7.2.2.8 Safety Profile:

Likely	Less Likely	Rare But Serious
• Diarrhea • Nausea • Vomiting • Constipation • Fewer white blood cells, red blood cells and platelets in the blood. • A low number of white blood cells can make it easier to get infections • A low number of red blood cells can make you feel tired and weak • A low number of platelets causes you to bruise and bleed more easily • Fever including fever with a low white blood cell count which could indicate infection and may require hospitalization and treatment with antibiotics • Pain which may be in your abdomen, back or bones • A feeling of weakness and/or tiredness • Temporary hair loss	• Loss of appetite • Elevation in the blood of certain enzymes or bilirubin found in the liver which could indicate liver irritation or damage • Headache • Rash, hives, itching or a red bumpy rash • A mild lowering of the blood pressure which usually does not require treatment • Inflammation and/or sores in the mouth, throat and/or esophagus • An infection in the blood which will require admission to the hospital and treatment with antibiotics • Numbness and tingling in the fingers and toes • Small amount of blood and/or protein in the urine or an elevation in blood creatinine which may indicate mild kidney damage • Shortness of breath • Muscle or joint aches and pains • Chest pain • Shaking chills	• Severe allergic reaction which can be life threatening with shortness of breath, low blood pressure and a rapid heart rate • Severe allergic reaction which can be life threatening with rapid build-up of fluid under the skin, in the lining of the intestine and possibly in the throat or swelling of the tongue which could make it difficult to breath. • Bleeding into the tumor which may cause damage depending on the location of the tumor.

7.3 Concomitant Medications

Low-dose aspirin (≤ 325 mg/d) may be continued in subjects at higher risk for arterial thromboembolic disease. Subjects developing signs of arterial ischemia or bleeding on study should be evaluated for possible bevacizumab discontinuation per Table 1, Bevacizumab Dose Management Due To Adverse Events.

All concomitant medications administered within 14 days prior to and during the active study period are to be reported on the appropriate case report forms for each patient.

7.3.1 Prior Treatment:

Reasonable efforts will be made to collect information on all prior cancer treatments received by the patient (chemotherapy, radiotherapy, immunotherapy, biologics, etc.). The information must be recorded in the patient's medical chart and on the patient's CRF.

All concomitant medications will be recorded on the appropriate case report forms.

7.3.2 Permitted Treatment:

All routine and appropriate supportive care will be provided during this study, as clinically indicated, and in accordance with the standard of care practices. Clinical judgment should be utilized in the treatment of any adverse event experienced by the patient.

Patients should receive full supportive care, including hematopoietic growth factors, transfusion of blood and blood products, aspirin, antibiotics, anti-emetics, as clinically appropriate**.** Pre-medication (anti-emetics, hydration, antihistamines, etc.) should be administered according to institutional standards. Pneumocystis carinii prophylaxis is strongly recommended.

Anti-platelet therapy (e.g. < 325 mg/day aspirin) should be considered for treatment of patients at high risk of developing arterial thromboembolic disease unless contraindicated. Low-dose aspirin (≤ 325 mg/d) may be continued in subjects at higher risk for arterial thromboembolic disease and are receiving aspirin at the time of study entry. Subjects developing signs of arterial ischemia or bleeding on study should be evaluated for possible bevacizumab discontinuation per Table 1, Bevacizumab Dose Management Due To Adverse Events.

Patients may continue or be placed on oral contraceptives or hormone-replacement therapy as clinically indicated.

Patients who experience infusion-associated symptoms may be treated symptomatically with acetaminophen, diphenhydramine, ranitidine, or other medications as clinically indicated.

Patients with indwelling venous catheters may receive prophylaxis against catheter thrombosis in accordance with the local standard of care. Because bevacizumab has a half-life of approximately 21 days, elective major surgery with the exception of bone marrow biopsies should be delayed whenever possible. No data are available to define a safe interval. Re-initiation of bevacizumab following surgery requires documented approval from the Principal Investigator and Sponsor.

Other medications considered necessary for the patient's safety and well-being may be given at the discretion of the investigator.

Information on all concomitant medications, blood products administered, as well as interventions occurring during the study period must be recorded on the patient's CRF.

8.0 CLINICAL AND LABORATORY EVALUATIONS

8.1 Pre-Treatment Evaluations

Informed consent must be obtained before study-specific screening evaluations are performed, unless performed as standard of care. The informed consent process should be documented in the patient's chart.

The following procedures or evaluations will be performed within 7 days prior to treatment with the exception of screening radiologic studies appropriate for the disease which will be performed within 14 days of the start of treatment:

- Complete medical and surgical history, including demographic data
- Prior cancer history (including all prior therapy)
- Complete physical examination
- Vital Signs (temperature, heart rate, blood pressure and respiratory rate)
- Lansky or Karnofsky performance status
- Height and weight
- Neurologic examination in patients with CNS tumors only
- Laboratory studies
    - CBC (WBC with differential, hemoglobin, hematocrit, and platelets)
    - Serum chemistries (albumin, sodium, potassium, chloride, bicarbonate, BUN, creatinine, glucose, calcium, total bilirubin, total protein, AST, ALT, alkaline phosphatase, phosphorus, and LDH)
    - Serum pregnancy test (for all females of childbearing potential) as per institutional guidelines
    - INR
    - Urine protein: creatinine ratio or urine dipstick (and 24 hour collection if indicated)
- Radiologic studies appropriate for disease assessment (CT, MRI, MIBG, etc.)
- EKG, echocardiogram, MUGA (as indicated)
- CSF cell count and cytology if appropriate for disease assessment
- Bone marrow aspiration(s) or biopsy if appropriate for disease assessment
- To determine the impact of bevacizumab on growth and development in children. Baseline assessment of growth and development will consist of bone age films (left hand) and measurement of somatomedin C levels at baseline and at designated times during treatment. The evaluation will be conducted in all patients except for females who are post menarche or males who are Tanner Stage V.
- Monitoring of protocol specified adverse events
- Recording of concomitant medications

8.2 Evaluations During Treatment

The following procedures or evaluations will be performed weekly during treatment except where designated in Table 2: Required Observations:

- Complete medical and surgical history, including demographic data
- Complete physical examination
- Vital Signs (temperature, heart rate, blood pressure and respiratory rate). Blood pressure monitoring will be performed weekly.
- Lansky or Karnofsky performance status
- Height and weight (to be collected prior to each cycle of treatment)
- Neurologic examination every cycle as clinically indicated in patients with non-CNS tumors.
- Laboratory studies
    - CBC (WBC with differential, hemoglobin, hematocrit, and platelets)
    - Serum chemistries (albumin, sodium, potassium, chloride, bicarbonate, BUN, creatinine, glucose, calcium, total bilirubin ,total protein, AST, ALT, alkaline phosphatase, phosphorus, and LDH)
    - Serum pregnancy test before each cycle (for females of childbearing potential only)
    - INR (for patients on warfarin only)
    - Urine protein: creatinine ratio or urine dipstick (and 24 hour collection if indicated) if indicated but otherwise every other dose. Screening urine protein cannot substitute for the day 0 time point, as the reason for the test is to see if there is a change from baseline, after bevacizumab has been given.
- Radiologic studies appropriate for disease assessment (CT, MRI, MIBG, etc.) after every 2 cycles of therapy.
- EKG, echocardiogram, MUGA (as indicated)
- Monitoring of protocol specified adverse events
- Recording of concomitant medications
- To determine the impact of bevacizumab on growth and development in children. Baseline assessment of growth and development will consist of bone age films (left hand) and measurement of somatomedin C levels at baseline, 6 months and the end of treatment.

8.3 Post-Treatment Evaluations / Off Study

The following procedures or evaluations will be performed at the completion of study participation:

- Complete medical and surgical history, including demographic data

- Complete physical examination
- Vital Signs (temperature, heart rate, blood pressure and respiratory rate)
- Lansky or Karnofsky performance status
- Height and weight
- Neurologic examination for patients with CNS tumors only
- Laboratory studies
    - CBC (WBC with differential, hemoglobin, hematocrit, and platelets)
    - Serum chemistries (albumin, sodium, potassium, chloride, bicarbonate, BUN, creatinine, glucose, calcium, phosphorus, total bilirubin ,total protein, AST, ALT, alkaline phosphate, phosphorus, and LDH)
    - INR (for patients on warfarin)
    - Urine protein: creatinine ratio or urine dipstick (and 24 hour collection if indicated)
- Radiologic studies appropriate for disease assessment (CT, MRI, MIBG, etc.)
- EKG, echocardiogram, MUGA (as indicated)
- Bone aspiration(s) or biopsy if appropriate for disease assessment
- Monitoring of protocol specified adverse events
- Recording of concomitant medications

8.4 Study Termination Visit

A study termination visit should be scheduled for ~30 days (28–42 days) after the decision to discontinue study participation. The following evaluations and procedures will be performed at this visit:

- Complete medical and surgical history, including demographic data
- Complete physical examination
- Vital Signs (temperature, heart rate, blood pressure and respiratory rate)
- Lansky or Karnofsky performance status
- Height and weight
- Monitoring of protocol specified adverse events

  Recording of concomitant medications

8.5 Overall Survival (OS) and Time To Progression (TTP)

Patients who complete or discontinue treatment will be followed for overall survival for up to 5 years and/or to the time of progression. This information will be requested by the Protocol Principal Investigator/POETIC Data and Coordinating Center via Email/Phone annually. Completion of case report forms is not required.

TABLE 2: REQUIRED OBSERVATIONS

	Pre-Study	Wk 1	Wk 2	Wk 3	Wk 4	Wk 5	Wk 6	Wk 7	Wk 8	Wk 9	Wk 10	Wk 11	Wk 12	Off Study	Study Termination Visit
Bevacizumab[a]		X			X			X			X				
Cyclophosphamide[b]		X			X			X			X				
Topotecan[b]		X			X			X			X				
Informed consent	X														
Demographics	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Medical history	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Concurrent meds	X	X----------X												X	X
Physical exam	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Vital signs (temp, HR, RR esp. BP)	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Height/Weight	X	X			X			X			X			X	X
Performance Status	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
CBC w/diff, platelets	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
INR (For all weeks except baseline, collect only for Warfarin patient)	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Serum chemistry[c]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
EKG, ECHO, MUGA (as indicated)															
CSF (as indicated)	X														
B-HCG	X[d]				X[d]			X[d]			X[d]				
Adverse event evaluation	X	X----------X												X	X
Tumor measurements	X	Tumor measurements are repeated every ≥ 4 and < 6 weeks. Documentation (radiologic) must be provided for patients removed from study for progressive disease.												X[e]	
Radiologic evaluation	X	Radiologic measurements should be performed every ≥ 4 and < 6 weeks.												X[e]	
Pharmacokinetics		X[f]	X	X	X	X	X								
Blood for angiogenic profile and serum angiogenic biomarkers		X[g]													
Somatomedin C	X[i]													X	
Bone age films	X[i]													X	
Blood for Metabolic Signature Analysis		X[h]	X	X											
Urine protein: creatinine ratio or dipstick	X	X						X					X	X	

a: Bevacizumab: Administered on Day -3 of every 21 day cycle
b: Cyclophosphamide/Topotecan: Administered on Days 0- 4 of every 21 day cycle
c: Albumin, alkaline phosphatase, total bilirubin, bicarbonate, BUN, calcium, chloride, creatinine, glucose, LDH, phosphorus, potassium, total protein, SGOT [AST], SGPT [ALT], sodium.
d: Serum pregnancy test will be preformed at the beginning of each cycle (Females of childbearing potential).
e: Off-study evaluation.
f: Pharmacokinetic Analysis: Samples will be collected pre and post treatment. See section 8.7.1 for schedule.
g: Angiogenic Profile and Serum Biomarkers: Day -3 (prior to administration of bevacizumab), Day 0 (prior to administration of cyclophosphamide), Day 7 and Day 18 (prior to the next scheduled dose of bevacizumab).
h: NMR analysis: Day -3 (prior to administration of bevacizumab), Day 0 (prior to administration of cyclophosphamide), and Day 7 of the first cycle of therapy .
i: Bone age films and somatomedin C will be obtained at base line, every 6 months thereafter, and the end of treatment.

Evaluations in patients who experience treatment delays

In the event of a treatment delay, the timetable for the evaluations above will be modified so that studies are performed at the times appropriate relevant to drug administration. Height, weight, vital signs, and performance status will be checked and documented during weeks in which drug is administered. Physical examinations will also be performed and documented during weeks in which drug is administered. Laboratory studies as shown in the table will be performed on a weekly basis during weeks in which drug is administered and during the following rest week. In the event of treatment delay, laboratory studies will be performed at additional time-points as needed for good patient care. Radiographic assessments will be performed after every 2 cycles of therapy.

8.6 Criteria For Therapeutic Response/Outcome Assessment

Patients with measurable disease will be assessed by standard criteria. For the purposes of this study, all patients with solid tumors will be re-evaluated every 2 cycles (≥ 4 and < 6 weeks from the start of the first cycle of treatment). In addition to a baseline scan, confirmatory scans will also be obtained 3 weeks following initial documentation of an objective response. If a durable response is documented, response will be re-assessed every 6 weeks**.** If treatment is delayed the radiologic imaging will be performed after every other cycle (which will result in a longer interval between imaging)**.**

8.6.1 Definition of Response in Patients

Response and progression will be evaluated in this study using the international criteria Revised RECIST guideline (version 1.1) proposed by the Response Evaluation Criteria in Solid Tumors (RECIST) Committee [European Journal of Cancer 45:228-247, 2009]. 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.

8.6.1.1 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).

8.6.1.2 Non-measurable Disease

All other lesions (or sites of disease), including small lesions (longest diameter < 20 mm with conventional techniques or <10 mm using spiral CT scan), are considered non-measurable disease. Bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusions, lymphangitis cutis/pulmonis, inflammatory breast disease, abdominal masses (not followed by CT or MRI), and cystic lesions are all non-measurable.

8.6.1.3 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 a reference by which objective tumor response might be characterized.

8.6.1.4 Non-target Lesions

All other lesions (or sites of disease) should be identified as non-target lesions and should also be recorded at baseline. Non-target lesions include measurable lesions that exceed the maximum numbers per organ or total of all involved organs as well as non-measurable lesions. Measurements of these lesions are not required, but the presence or absence of each should be noted throughout follow-up.

8.6.1.5 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 and never more than 14 days before the beginning of the treatment. Tumors in a previously irradiated area will be considered measurable.

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 antitumor 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. Tumors of the head, neck and extremities usually require specific protocols.

Ultrasound (US). When the primary endpoint of the study is objective response evaluation, 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.

Endoscopy, Laparoscopy. The utilization of these techniques 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 reference centers. However, such techniques can be useful to confirm complete pathological response when biopsies are obtained.

Cytology, Histology. These techniques can be used to differentiate between partial responses (PR) and complete responses (CR) in rare cases (e.g., residual lesions in tumor types such as germ cell tumors, where known residual benign tumors can remain).

Because an effusion may be a side effect of treatment, cytological confirmation of the neoplastic origin of any effusion that appears or worsens during treatment (when the measurable tumor has met criteria for response or stable disease) is mandatory to differentiate between response, stable disease and progressive disease.

8.6.2 Response Criteria

8.6.2.1 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 longest diameter (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.

8.6.2.2 Evaluation of Non-target Lesions

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

Incomplete Response/Persistence of one or more nontarget lesion(s).

Stable Disease (SD): 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.

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 at a later time by the review panel (or study chair).

If tumor markers are initially above the upper normal limit, they must normalize for a patient to be considered in complete clinical response.

8.6.3 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 progressive disease the smallest measurements recorded since the treatment started). The patient's best response assignment will depend on the achievement of both measurement and confirmation criteria.

Target Lesions	Non-target Lesions	New Lesions	Overall Response
CR	CR	No	CR
CR	Incomplete response/SD	No	PR
PR	Non-PD	No	PR
SD	Non-PD	No	SD
PD	Any	Yes or No	PD
Any	PD	Yes or No	PD
Any	Any	Yes	PD

Note: 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) before confirming the complete response status.

To be assigned a status of PR or CR, changes in tumor measurements must be confirmed by repeat evaluations (CT/MRI) 3 weeks after the criteria for response are first met.

8.6.4 Confirmatory Measurement/Duration of Response

8.6.4.1 Confirmation

To be assigned a status of PR or CR, changes in tumor measurements must be confirmed by repeat assessments obtained 3 weeks after the criteria for response are

first met. In the case of SD, follow-up measurements must meet the SD criteria at least once after study entry at a minimum interval of 6 weeks.

8.6.4.2 Duration of Overall Response

The duration of overall response is measured from the time measurement criteria are met for CR or PR (whichever is first recorded) until the first date that recurrent or progressive disease is objectively documented (utilizing as reference for progressive disease the smallest measurements recorded since the treatment started).

The duration of overall CR is measured from the time measurement criteria are first met for CR until the first date that recurrent disease is objectively documented.

8.6.4.3 Duration of Stable Disease

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

8.7 Correlative Studies

The study will incorporate mandatory exploratory biologic correlative studies to evaluate the following:

8.7.1 Pharmacokinetic Analysis

These studies will be conducted under the direction of Dr. Aru Narendran at the University of Calgary in Alberta, Canada.

Serum samples will be obtained before and at the end of each bevacizumab infusion. Pharmacokinetic serum samples will be obtained during the first and second cycles of treatment and at the first follow-up visit for response assessment.

Cycle 1 (21 day cycle)

Day -3 (Bevacizumab Dose 1): pre and immediate post infusion sample
Day 7 ± 3 days: Trough
Day 18 ±3 days: Trough

Cycle 2 (21 day cycle)

Day 7 ± 3 days: Trough
Day 18 ± 3 days: Trough

Trough at follow-up for the initial response assessment at 6 weeks after the start of treatment.

Blood should be drawn from the contralateral arm to the infusion site using an indwelling catheter to avoid multiple needle sticks. If a catheter is used for blood collection, the fluid in the catheter should be completely withdrawn prior to sample collection and discarded. About 5 mL of blood will be collected in a red top tube at each time point, placed upright (30 minutes) and centrifuged 3,000 x *g* for 10 minutes at 4°C. Serum will be collected and stored at –70°C until analysis.

Samples should be batched and shipped to the following address:

Aru Narendran, MD, PhD
University of Calgary
HRIC Building, Lab 2a34
3280 Hospital Drive NW
Calgary, Alberta T2N 4Z6
CANADA
Telephone: (403) 210-6402
Fax: (403) 210-6418
E-mail: anarendran@ucalgary.ca

Bevacizumab concentrations will be determined using an enzyme-linked immunosorbent assay (ELISA) established by Genentech.

8.7.2 Assessment of Angiogenic Profile

In order to assess the angiogenic profile in patients enrolled in this study, the following analyses will be performed:

Measurement of EPC and HPC

Blood volumes collected will be based on the patient's weight as outlined in the Procedural Manual (page 5). Blood will be collected in Cycle 1 only at the following time-points: Day -3 (prior to bevacizumab therapy), Day 0 (prior to chemotherapy) and 7, and prior to next scheduled dose of bevacizumab. Peripheral blood will be collected in an EDTA tube (purple top tube) and placed on ice. Specimens should be collected Monday through Thursday to allow for shipping and processing. Blood will be centrifuged to collect plasma and then the cellular portion will be diluted and placed on a Ficoll-Opaque gradient to obtain the mononuclear cell layer. These cells will be stained for VEGFR1, VEGFR2, VEGFR3 along with stem/progenitor markers CD34, CD133 and cKIT. HPCs will be defined as $CD34^+$ $VEGFR1^+$ cells and EPCs will be defined by VEGFR2 and CD133. 7-AAD staining will be used to assess the number of apoptotic cells. Mononuclear cells will also be collected and placed in 100 μl lysis solution for RNA extraction and quantification of mRNA levels in these circulating mononuclear cells.

Furthermore, we will perform CD34 enrichment by using CD34 microbeads for flow cytometry and to obtain CD34 enriched population for RNA analysis in order to perform microarray analysis on $CD34^+$ cells in pediatric lymphoma patients in order to better characterize this cell population.

We also plan to measure VEGF-A, VEGF-B (signals through VEGFR1 alone), VEGF-C, PlGF (signals through VEGFR1 alone), FGF (fibroblast growth factor important in fibroblast activation and production of fibronectin the platform for circulating HPCs expressing the integrin VLA-4), SDF-1 (stromal derived factor-1 known to mobilize HPCs from the bone marrow), HGF/SC (hepatocyte growth factor/ scatter factor known to be elevated in many cancer types and plays a particularly important role in tumor invasion and spread) from the platelet-enriched plasma. The plasma will be obtained after centrifugation of blood at 2000 rpm for 20 minutes. The collected plasma portion will be spun again at 6000 rpm in order to obtain the platelet enriched fraction.

If bone marrow cells are obtained during the course of treatment, similar studies to the blood studies will be performed on these cells as well as culturing of bone marrow lineage depleted cells in megakaryocyte specific chemokines in order to obtain profiling of megakaryocytes which are key regulators of VEGF storage and release in the bone marrow microenvironment. The methodology as noted above for the blood samples will also be used for the bone marrow samples.

In addition to ELISA quantification of VEGF-A, VEGF-B and PlGF levels in plasma, Western blots will be performed for free VEGF and a non-denaturing gel will be used to evaluate VEGF bound to bevacizumab.

Contact Information for Sample Shipment:

David C. Lyden MD, PhD
Stavros S. Niarchos Professor
Department of Pediatrics, and Cell and Developmental Biology
Weill Medical College of Cornell University
413 E. 69th Street, Box 284
New York, NY 10021
Office: 646-962-6238
Fax: 646-962-0574
E-mail: dcl2001@med.cornell.edu

8.7.3 Expression of Angiogenesis Associated Serum Biomarkers

Plasma samples will be collected from patients before and after the initiation of treatment at scheduled intervals according to the treatment schema. Samples will be analyzed for levels of VEGF, bFGF, Ang-1, Ang-2, sFlt-1, sTie-2, and PlGF using commercially available ELISA kits (R&D Systems, Minneapolis, MN). Each sample will be assayed in triplicate. Technical steps will be carried out according to the instructions supplied by the manufacturer. Data will be analyzed using GraphPad Prism version 3.00 statistical software (GraphPad Software, San Diego, CA). Correlative analysis will be carried out with clinical parameters indicated in the protocol such as tumor type, grade, adverse events and treatment response.

Five mL of peripheral blood drawn will be collected in EDTA containing tubes (purple top tube) in cycle 1 only at the following time-points: Day -3 (prior to administration of bevacizumab), Day 0 (prior to administration of cyclophosphamide), Day 7 and Day 18 (prior to the next scheduled dose of bevacizumab) and centrifuged at 1200 rpm for approximately 10 minutes. Transfer collected plasma to Eppendorf tubes (maximum 1 ml per tube) and store at -20^{0}C until shipment on dry ice. Samples can be batched and shipped. Please send samples by overnight only on Monday, Tuesday or Wednesday. If a sample to be collected on any other day, it can be kept in a -20^{0}C freezer until shipment. Please enclose the information for Canada Customs (attached).

If possible, E-mail the shipment information to anarendran@ucalgary.ca and karen.mazil@albertahealthservices.ca.

The shipping address is as follows:

Dr. Aru Narendran
University of Calgary
HRIC Building, Lab 2a34
3280 Hospital Drive NW
Calgary, Alberta T2N 4Z6
CANADA
Telephone: (403) 210-6402
(403) 210-6418

8.7.4 Metabolic Signature Analysis through Magnetic Resonance Spectroscopy

Identification of the metabolic changes exhibited by cancer cells in response to a therapeutic agent can provide essential information with respect to drug activity as well as toxicity. In these experiments we will attempt to identify changes in metabolite levels in tumor tissues in response to the proposed treatment. The principles of the methodology have been reviewed previously.[70] Briefly, aqueous metabolites will be extracted and proteins will be precipitated using a chloroform:methanol system (method of Bligh and Dyer) and an untargeted gas chromatography mass spectrometry (GC-MS) analysis will be performed following chemical derivatization. Multivariate statistical methods, namely principal component analysis (PCA) and partial least squares discriminant analysis (PLS-DA) will be performed to determine the clustering properties of the samples. Database matches will then be done to determine the identities of the most significant metabolites. Finally, the identities of the metabolites that change in response to treatment will be documented and analyzed.

Tissue for Metabolic NMR Spectroscopy

Optimal sample size is 50 mg of tissue *No formalin or other cold preservatives must be used except for the use of liquid nitrogen. Specimens must be harvested in the operating room or biopsy procedure room, with liquid nitrogen on site.

1. Promptly remove the tissue of interest.
2. Immediately place it in liquid nitrogen.
3. Store frozen samples at -80 degrees Celsius.
4. When a sample set is ready to be shipped for analysis, please ship the samples via FedEx (overnight) on sufficient dry ice to allow for 48 hours of storage. Ship only on Mondays, Tuesdays, or Wednesdays to avoid weekend delays. See below for shipping information.

Human Whole Blood Collection for Metabolic NMR Spectroscopy

Only **heparin-**preserved blood can be used for NMR analysis (please, no EDTA and no citrate coating). The heparin tubes have green tops (sodium heparin) 5 mL whole blood is required and will be collected at the following time-points: Day -3 (prior to bevacizumab administration), Day 0 (prior to cyclophosphamide administration), and Day 7 of the first cycle of therapy.

1. Collect minimum of 5 mL venous blood into a heparin-coated tube.
2. Shake / vortex well.
3. Immediately after collection, place the tube on ice for ~10 minutes.
4. Store samples at -20 or -80 degrees Celsius. Samples can be batched.

5. When a sample set is ready to be shipped for analysis, please ship the samples via FedEx (overnight) on sufficient dry ice to allow for 48 hours of storage. Ship only on Mondays, Tuesdays or Wednesdays to avoid weekend delays. See below for shipping information.

Shipping Instructions

At least 24 hours prior to shipping samples, please contact Dr. Aru Narendran at the telephone number below and e-mail (anarendran@ucalgary.ca.) to inform about upcoming sample shipment. Please provide (via email or inside the FedEx box) the exact sample study numbers/ identification, including the patient study number, source of sample, date and time of sample collection.

SHIP SAMPLES ONLY ON MONDAYS, TUESDAYS, OR WEDNESDAYS.

Contact Information for Sample Shipment*:*

Dr. Aru Narendran
University of Calgary
HRIC Building, Lab 2a34
3280 Hospital Drive NW
Calgary, Alberta T2N 4Z6
CANADA

Telephone: (403) 210-6402
(403) 210-6418

8.7.5 Assessment of Impact of Bevacizumab on Growth and Development

Baseline assessment of growth and development will consist of bone age films (left hand) and measurement of somatomedin C levels at baseline, 6 months and at the time that the patient is taken off study. The most useful information will be obtained from patients who have durable responses.

9.0 SUBJECT DISCONTINUATION

Subjects who meet the following criteria should be discontinued from study treatment:

- Grade 4 hypertension or Grade 3 hypertension not controlled with medication
- Nephrotic syndrome
- Grade 1 pulmonary or CNS hemorrhage for patients who are receiving full-dose anticoagulation.
- Grade ≥ 2 pulmonary or CNS hemorrhage; any Grade 4 hemorrhage
- Symptomatic Grade 4 venous thromboembolic event (for lung protocols: any venous thromboembolic event requiring full dose warfarin or equivalent (i.e., unfractionated or low molecular weight heparin)

- Any grade arterial thromboembolic event
- Grade 4 congestive heart failure
- Gastrointestinal perforation
- Tracheoesophageal fistula (any grade) or Grade 4 fistula
- Bowel obstruction that has not fully recovered despite medical or surgical intervention
- Wound dehiscence requiring medical or surgical intervention
- Unwillingness or inability of subject to comply with study requirements
- Determination by the investigator that it is no longer safe for the subject to continue therapy
- All Grade 4 events thought to be related to bevacizumab by the investigator

Patients who have an ongoing bevacizumab-related Grade 4 or serious adverse event at the time of discontinuation from study treatment will continue to be followed until resolution of the event or until the event is considered irreversible (see Section 7.1.3).

10.0 STUDY DISCONTINUATION

Genentech has the right to terminate this study at any time. Reasons for terminating the study may include, but are not limited to, the following:

- The incidence or severity of adverse events in this or other studies indicate a potential health hazard to patients.
- Patient enrollment is unsatisfactory.
- Data recording is inaccurate or incomplete.

11.0 STATISTICAL METHODS

11.1 Determination of Sample Size

It is anticipated that 1-2 patients per month will be accrued on this protocol. Based on these estimates, a total of 29 patients will be enrolled on study with patient accrual completed in approximately 1 ½ years.

11.2 Stratification of Accrual

To ensure that both disease groups are well represented, the accrual will be stratified. This measure will allow additional information about the response rates of each individual group to be obtained, however the success or failure of the combination, CTB, will be assessed based upon composite data from both disease groups combined.

11.3 Planned Efficacy Evaluations

11.3.1 Primary Efficacy Variables

The primary efficacy variables are objective response rate after 2 cycles of treatment and duration of response. Patients who experience early progression of disease prior to receiving the second cycle of therapy will be considered a treatment failure.

11.3.2 Secondary Efficacy Variables

The secondary efficacy variables include time to progression The planned length of follow-up will be up to 5 years or until disease progression (Please refer to section 8.5 for data requirements)

11.3.3 Methods of Analysis

In previous trials[69], conducted in this population, the response rate (CR+ PR) for this patient population was approximately 0.30. A minimax Simon 2-stage design that differentiates between population response rates of 0.30 and 0.55 will be used. A total of 29 patients will be accrued onto this study. We will enroll 14 patients in the first stage, 7 patients from each disease group. If 4 or fewer responses are observed, then the trial is stopped. In that case, if there are at least 3 responses in one disease group, we will consider studying this treatment in another phase II study limited to that disease group. If at least 5 responses are observed, then 15 additional patients will be enrolled onto the study with at least 7 patients accrued from each disease group, for a total of 29 patients. In this scenario, even if all 5 responses are seen in one disease group, accrual will continue; however, this outcome is not likely even if the treatment is effective in only one group. (If the response rate in the neuroblastoma group is 55% and EWS 30%, the probability of having $\geq$ 5/7 responders in NB and 0/7 in EWS is 3%. If NB has 55% response rate and EWS has 20%, the probability is 7%.) At the conclusion of the trial, if 13 or more responses, the treatment will be considered successful. This design has power 0.88 for a population response proportion of 0.55 using a one-sided test with size 0.07.

At the conclusion of the study, overall survival and progression free survival probabilities over time will be computed using the Kaplan-Meier method. Subgroup response rates will be reported as well. In addition, a summary of the protein levels will be recorded at each time point, and the paired t-test or sign test will be used to determine if the pre and post protein levels within patient are equal.

11.4 Analysis of Correlative Studies

Pharmacokinetic Analysis

The pharmacokinetic parameters of bevacizumab (AUC, C_{max}, $t_{1/2}$) will be calculated using nonlinear mixed effects modeling (NONMEM, GloboMax® LLC, Hanover, MD).

Analysis of Angiogenic Profile

Blood samples will be collected at study entry and throughout the course of treatment as outlined above. Categorical data will be compared using Fisher's exact probability test and continuous data will be compared with Student's test if the sample distribution is normal or with Mann-Whitney U test if the sample distribution is asymmetrical. Kaplan-Meier life-table curves will be constructed to estimate survival free of local-regional and distant recurrences and overall survival. Data on patients who are alive and without evidence of disease at the end of five-year study will be considered censored. The comparison between survival functions for different strata will be assessed with the log-rank statistics.. Differences will be considered significant when $P < 0.05$. Using these statistical methods, we will compare circulating HPC levels with treatment response and disease free survival. Cutoff values of 'positive' circulating HPC levels will be established in order to test these values for discriminating power in predicting disease

outcome. Once cutoffs are established as it has been in other tumor types, these values will be used to establish correlation with disease outcome.

Analysis of Expression of Angiogenesis Associated Serum Biomarkers

Each sample will be assayed in triplicate. Technical steps will be carried out according to the instructions supplied by the manufacturer. The quantity of individual cytokines (pg/ml) found in each sample and control normal plasma (n=20, investigator's collection) will be analyzed as follows. The Wilcoxon signed ranks test or paired t-test will be used to compare pre and post treatment protein levels as appropriate. To examine differences between patients and controls, and at various time points with respect to treatment, Wilcoxon-Mann Whitney test or Student's t-test will be used. The Spearman rank correlation test will be used to assess whether the levels of the measured cytokines correlated with clinical parameters indicated in the protocol such as tumor type, grade, adverse events and treatment response. All analyses will be performed by using SAS version 9.1, and, P-values less then .05 will be considered to be statistically significant.

Analysis of Metabolic Profiling

A descriptive analysis will be used to analyze the data obtained from the metabolic profiling analysis based upon the pattern of MRS.

Analysis of Impact of Bevacizumab on Growth and Development

A descriptive analysis will be used to describe the effect of bevacizumab on growth and development based upon the results of the serial measurements of Somatomedin C and bone age films.

12.0 SAFETY REPORTING OF ADVERSE EVENTS

12.1 Adverse Event Reporting and Definitions

In the event of an adverse event the first concern will be for the safety of the subject.

The protocol principal investigator/ POETIC DCC are required to report to Genentech Drug Safety ANY serious treatment emergent adverse event (STEAE) as soon as possible.

A STEAE is any sign, symptom or medical condition that emerges during Bevacizumab treatment or during a post-treatment follow-up period that (1) was not present at the start of Bevacizumab treatment and it is not a chronic condition that is part of the patient's medical history, OR (2) was present at the start of Bevacizumab treatment or as part of the patient's medical history but worsened in severity and/or frequency during therapy, AND that meets any of the following regulatory serious criteria:

- Results in death
- Is life-threatening
- Requires or prolongs inpatient hospitalization
- Is disabling
- Is a congenital anomaly/birth defect
- Is medically significant or requires medical or surgical intervention to prevent one of the outcomes listed above.

The following events will not be considered reportable:

- Grade 3 or 4 Neutropenia for a duration < 7 days
- Grade 3 or 4 Leukopenia for a duration < 7 days
- Lymphopenia
- Grade 3 or 4 Thrombocytopenia for a duration < 7 days
- Anemia

12.2 Reporting of Serious Treatment Emergent Adverse Events

The Protocol Chairman is responsible for monitoring the safety of patients who enroll in the study. All AEs and SAEs occurring after consent has been signed and after any administration of the study drug regardless of drug attribution will be followed to the end of the study including 30 days after the last administration of study drug, as well as any SAEs designated possibly, probably or definitely related to treatment that occur greater than 30 days.

The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be used for adverse event and serious adverse event reporting. All participating sites should have access to a copy of the CTCAE version 4.0. A copy of the CTCAE version 4.0 can be downloaded from the CTEP web site (http://ctep.cancer.gov/reporting/ctc.html).

The Protocol Chairman is required to report all adverse events that occur during the clinical study starting when the patient signs consent throughout 30 days of stopping the investigational agent. Severe adverse events must be reported to the appropriate protocol-defined study sponsors. Serious adverse events must be reported by email and/or telephone to the Protocol Chairman, Data and Coordinating Center at Memorial Sloan-Kettering Cancer Center, and local IRB within 24 hours of knowledge of their occurrence. A written SAE report including source documentation must be sent to the Protocol Chairman and Data and Coordinating Center within another 3 calendar days, using the Serious Adverse Event case report form and MedWatch 3500a form.

Additionally, the Protocol Chairman is responsible for submitting follow-up reports for all SAEs regarding the patient's subsequent course until the SAE has resolved or until the patient's condition stabilizes (in the case of persistent impairment), or the patient dies.

In the event of an adverse event the first concern will be for the safety of the subject.

Investigators are required to report to the DCCANY serious treatment emergent adverse event (STEAE) as soon as possible. The Protocol Chairman is responsible for reporting such STEAEs to Genentech Drug Safety.

Reporting Requirements for Adverse Events That Occur on Treatment and Within 30 Days[1] of the Last Dose of Study Drug

	Grade 1	Grade 2		Grade 2		Grade 3		Grade 3		Grades 4 & 5
	Unexpected and Expected with or with-out hospitalization	Unexpected		Expected		Unexpected		Expected		Unexpected and Expected
		With Hospitalization	Without Hospitalization	With Hospitalization	Without Hospitalization	With Hospitalization	Without Hospitalization	With Hospitalization	Without Hospitalization	
Unrelated Unlikely	**Not Required**	**SAE Report Required**	**Not Required**	**SAE Report Required**	**Not Required**	**SAE Report Required**	**Not Required**	**SAE Report Required**	**Not Required**	**SAE Report Required**
Possible Probable Definite	**Not Required**	**SAE Report Required**	**SAE Report Required**	**SAE Report Required**	**Not Required**	**SAE Report Required**	**SAE Report Required**	**SAE Report Required**	**Not Required**	**SAE Report Required**

[1] **Adverse events with attribution of possible, probable, or definite that occur <u>greater</u> than 30 days after the last dose of study treatment require an SAE report as follows:**

- Grade 3 unexpected events with hospitalization or prolongation of hospitalization
- Grade 4 unexpected events
- Grade 5 expected events and unexpected events

Initial notification for all STEAEs must include:

- Grade of event
- Date of event
- A brief description of the event
- Attribution to the protocol defined drug combination
- Patient Status

Relationship of any adverse event to protocol defined drug combination should use the following criteria:

- Definite - The adverse event *is clearly related* to the drug combination
- Probable - The adverse event *is likely related* to the drug combination.
- Possible - The adverse event *may be related* to the drug combination.
- Unlikely - The adverse event *is doubtfully related* to the drug combination.
- Unrelated - The adverse event *is clearly NOT related* to the drug combination.

STEAEs must be reported within 24 hours of the investigator's knowledge by phone or e-mail to:

- Data and Coordinating Center
- Lead Principal Investigator of the protocol/Protocol Chairman - (Dr. Tanya Trippett)
- Local IRB

STEAE contact information for the Data and Coordinating Center is listed below:

Memorial Sloan Kettering Cancer Center
Pediatric & Allo Transplant Clinical Trials Office
405 Lexington Avenue, room 3-512
New York, NY 10174
Phone: 646-888-5714/5715
Fax: 646-888-5726

Contact information for the Protocol Principal Investigator is listed below:

Tanya Trippett, MD
Memorial Sloan-Kettering Cancer Center
1275 York Avenue
New York, NY 10065
Telephone (212) 639-8267
Fax (212) 717-3239
E-mail Trippet1@mskcc.org

In the event of an AE, appropriate medical and supportive care will be administered. All efforts will be made to minimize the side effects and to support the patient until the toxicity resolves.

12.2.1 Reporting of STEAE to Sponsor

All STEAEs should be recorded on a MedWatch 3500a Form and faxed to:

Data and Coordinating Center/Principal Investigator
Tanya Trippett, MD
Fax: 646-888-5726

Reporting of STEAEs to the sponsor are to be handled by the Data and Coordinating Center at Memorial Sloan-Kettering Cancer Center. The DCC will fax all STEAEs to:

Genentech Drug Safety
Fax: (650) 225-4682 or (650) 225-5288

MedWatch 3500a Reporting Guidelines:

In addition to completing appropriate patient demographic and suspect medication information, the report should include the following information within the Event Description (section 5) of the MedWatch 3500a form:

- Treatment regimen (dosing frequency, combination therapy)
- Protocol description (and number, if assigned)
- Description of event, severity, treatment, and outcome, if known
- Supportive laboratory results and diagnostics
- Investigator's assessment of the relationship of the adverse event to each investigational product and suspect medication

Follow-up information:

Additional information may be added to a previously submitted report by any of the following methods:

- Adding to the original MedWatch 3500a report and submitting it as follow-up
- Adding supplemental summary information and submitting it as follow-up with the original MedWatch 3500a form

- Summarizing new information and faxing it with a cover letter including subject identifiers (i.e. D.O.B. initial, subject number), protocol description and number, if assigned, suspect drug, brief adverse event description, and notation that additional or follow-up information is being submitted (The subject identifiers are important so that the new information is added to the correct initial report)

Occasionally Genentech may contact the reporter for additional information, clarification, or current status of the subject for whom and adverse event was reported.

Assessing Causality:

Investigators are required to assess whether there is a reasonable possibility that bevacizumab caused or contributed to an adverse event. The following general guidance may be used.

Yes: If the temporal relationship of the clinical event to bevacizumab administration makes a causal relationship possible, and other drugs, therapeutic interventions or underlying conditions do not provide a sufficient explanation for the observed event.

No: If the temporal relationship of the clinical event to bevacizumab administration makes a causal relationship unlikely, or other drugs, therapeutic interventions or underlying conditions provide a sufficient explanation for the observed event.

12.3 Safety Reporting Requirements for IND Holders

For **Investigator Sponsored IND Studies**, there are some additional reporting requirements for the FDA in accordance with the guidance set forth in 21 CFR 312.32. Sponsor-investigators of studies conducted under an IND must comply with the following safety reporting requirements:

a. Expedited IND Safety Reports:

7 Calendar-Day Telephone or Fax Report:
The Sponsor-Investigator is required to notify the FDA of any fatal or life-threatening adverse event that is **unexpected and assessed by the investigator to be possibly related to the use of Bevacizumab**. An unexpected adverse event is one that is not already described in the Investigator Brochure. Such reports are to be telephoned or faxed to the FDA and Genentech within 7 calendar days of first learning of the event. Each telephone call or fax transmission (see fax number below) should be directed to the FDA new drug review division in the Center for Drug Evaluation and Research or in the product review division for the Center for Biologics Evaluation and Research, whichever is responsible for the review of the IND.

15 Calendar-Day Written Report:
The Sponsor-Investigator is also required to notify the FDA and all participating investigators, in a written IND Safety Report, of any serious, unexpected AE that is considered possibly related to the use of bevacizumab. An unexpected adverse event is one that is not already described in the Investigator Brochure.

Written IND Safety Reports should include an Analysis of Similar Events in accordance with regulation 21 CFR § 312.32. All safety reports previously filed with the IND concerning similar events should be analyzed. The new report should contain comments on the significance of the new event in light of the previous, similar reports.

Written IND safety reports with Analysis of Similar Events are to be submitted to the FDA, Genentech Drug Safety, and all participating investigators within 15 calendar days of first learning of the event. The FDA prefers these reports on a MedWatch 3500a Form but alternative formats are acceptable (e.g. summary letter).

FDA fax number for IND Safety Reports:

1 (800) FDA - 0178

All written IND Safety Reports submitted to the FDA by the Sponsor-Investigator must also be faxed to:

Genentech Drug Safety
Fax: (650) 225-4682 or (650) 225-5288
(Please use the safety reporting fax cover sheet attached to this document for your fax transmission)

AND:

Study Coordination Center/Principal Investigator
Tanya Trippett, MD
Fax: 646-888-5726

For questions related to safety reporting, contact:

Genentech Drug Safety
Tel: 1-888-835-2555
or
Fax: (650) 225-4682 or (650) 225-5288
(Please use the safety reporting fax cover sheet attached to this document for your fax transmission)

b. IND Annual Reports

In accordance with the regulation 21 CFR § 312.32, the Sponsor-Investigator shall within 60 days of the anniversary date that the IND went into effect submit a brief report of the progress of the investigation. Please refer to Code of Federal Regulations, 21 CFR § 312.32 for a list of the elements required for the annual report. All IND annual reports submitted to the FDA by the Sponsor-Investigator should be copied to Genentech. Copies of such reports should be mailed to:

Genentech, Inc.
ATTN: Avastin IST Coordinator
1 DNA Way, Mailstop 445-A
South San Francisco, CA 94080-4990
Tel: (650) 225-7121 (Oncology Hotline)

PLEASE NOTE: Section 12.4 is mandatory if your site has received an IND Exemption Letter from the FDA

12.4 Safety Reporting Requirements for IND Exempt Studies

For **Investigator Sponsored IND Exempt Studies**, there are some reporting requirements for the FDA in accordance with the guidance set forth in 21 CFR 314.80.

Postmarketing 15-Day "Alert Report":
The Sponsor-Investigator is required to notify the FDA of any fatal or life-threatening adverse event that is **unexpected and assessed by the investigator to be possibly related to the use of Bevacizumab**. An unexpected adverse event is one that is not already described in the Investigator Brochure. Such reports are to be submitted to the FDA (2 copies) at the following address: Central Document Room, 12229 Wilkins Avenue, Rockville, MD 20852.

All Postmarketing 15-Day "Alert Reports" submitted to the FDA by the Sponsor-Investigator must also be faxed to: Genentech Drug Safety

Fax: (650) 225-4682 or (650) 225-5288 (Please use the safety reporting fax cover sheet attached to this document for your fax transmission)

For questions related to safety reporting, contact:

Genentech Drug Safety
Tel: 1-888-835-2555
or
Fax: (650) 225-4682 or (650) 225-5288
(Please use the safety reporting fax cover sheet attached to this document for your fax transmission)

13.0 RETENTION OF RECORDS

U.S. FDA regulations (21 CFR 312.62[c] and the ICH Guidelines for GCP require that records and documents pertaining to the conduct of this study and distribution of the bevacizumab including CRFs, consent forms, laboratory test results, documentation of adverse events, medication inventory records, and all IRB correspondence must be retained by the Principal Investigator for at least 2 years after the investigation is completed.

14.0 RECRUITMENT PLAN/INFORMED CONSENT PROCEDURES

14.1 Patients will be offered the opportunity to participate in this trial if they meet all eligibility criteria. There will be no discrimination against females or minorities. Informed consent will be obtained from the patient, or if they are non-emancipated minors, their

parent or legal guardian. Consent will be obtained by an investigator authorized to obtain consent. Patients will not receive any payment for their participation in this study.

14.2 Patients or their parent/legal guardian will be required to sign an IRB approved statement of informed consent indicating the investigational nature of this study, indicating their consent to participate.

This consent form meets the requirements of the Code of Federal Regulations and the Institutional Review Board/Privacy Board of this Center. The consent form will include the following:

1. The nature and objectives, potential risks and benefits of the intended study.
2. The length of study and the likely follow-up required.
3. Alternatives to the proposed study. (This will include available standard and investigational therapies. In addition, patients will be offered an option of supportive care for therapeutic studies.)
4. The name of the investigator(s) responsible for the protocol.
5. The right of the participant to accept or refuse study interventions/interactions and to withdraw from participation at any time.

Before any protocol-specific procedures can be carried out, the consenting professional will fully explain the aspects of patient privacy concerning research specific information. In addition to signing the IRB Informed Consent, all patients must agree to the Research Authorization component of the informed consent form.

14.3 The investigators listed on the cover page and their qualified designees at each institution as listed on the FDA 1572 for this study may obtain consent and care for the patients according to good clinical practice and protocol guidelines.

14.4 One to three copies of the informed consent will be signed and dated by the patient, parent or the patient's legally authorized representative and by the physician obtaining informed consent, as per institutional guidelines. If institutional guidelines mandate that only one original copy is signed, the following sentences will refer to photocopies of the original. One copy will be given to the patient/parent/legal guardian to be retained for their personal records. One copy will be maintained on file at the Data and Coordinating Center. The third copy will be confidentially maintained by the participating institution.

14.5 A note will be placed in the medical record documenting that informed consent was obtained for this study, and that the patient and/or his/her parents, to the degree of their understanding, acknowledge and accept the risk of participation in this study.

14.6 Written consent must be documented on the appropriate consent form approved by the Institutional Review Board at the individual participating center. Attainment of the written consent must be verified by the Data and Coordinating Center (or equivalent entity) of the participating institution prior to entry on study. A copy of the consent form, along with the eligibility checklist, and Health Insurance Portability and Accountability Agreement (HIPAA) must be submitted to the Clinical Trials Office at the Data and Coordinating Center prior to enrollment of any subject on this study. Verification of subject enrollment on this study will be sent by email to the participating center immediately on receipt of the required documents.

14.7 Every effort will be made to include women, children of both sexes and minorities in the study population for this trial. No patient will be excluded from participation in this trial on the basis of gender, ethnicity, or race. Review of accrual to past pediatric multicenter studies of new agents demonstrates the accrual of both genders and all NIH-identified ethnicities to such studies. The small number of patients entered into this trial will obviate any analysis of variation in toxicity profile or response rate with gender or ethnicity.

Data from the institutions participating in this trial have been reviewed with respect to enrollment of patients of different races and genders. It is anticipated that enrollment of the current study will follow the same pattern.

Institution	Number of Patients	White Non-Hispanic (%)	Black Non-Hispanic (%)	Hispanic (%)	Other (%)
MSKCC	147	61.0	19.0	15.0	5.0
JHMC	77	73.0	21.0	1.0	5.0
UCS/TCH	204	62.0	0.0	34.0	4.0
MDACC	146	60.0	5.0	29.0	6.0
DFCI	250	81.3	4.7	0.5	9.2
PCH	137	65.7	< 0.1	27.7	6.5
CHM	184	71.0	12.0	13.0	4.0
MDACCO	90	50	9	26	15
PSH	85	81	6	0	13

15.0 RESEARCH PARTICIPANT REGISTRATION AND RANDOMIZATION PROCEDURES

15.1 Research Participant Registration

All patients must be centrally registered at the POETIC Data and Coordinating Center (DCC) located at Memorial Sloan-Kettering Cancer Center. Registrations will be handled by the RSA at the POETIC DCC. The contact telephone number is (646) 888-5714/5715 and the fax number is (646) 888-5726. Registrations will occur between 9:00 am and 5:00 pm Eastern Standard Time (EST), Monday through Friday and will include review of the signed consent form, HIPAA research authorization form, eligibility checklist, eligibility source documentation and Patient Enrollment form. The RSA will also verify, via a FAX/email copy, that the written informed consent is obtained and dated prior to subject entry on the study.

The participating sites must contact the Research Study Assistant (RSA) at the POETIC DCC and the Protocol Principal Investigator to reserve a slot on the protocol when a patient is being considered for a trial. To begin a registration the participating site must fax/email the signed consent form and HIPAA research authorization to the RSA within 48 hours of the patient signing consent. To complete the registration, the completed eligibility checklist, patient eligibility source documentation and the Patient Enrollment form must be faxed/emailed to the RSA at MSKCC prior to protocol treatment or any research tests.

All research participants are registered through the Protocol Participant Registration (PPR) Office at Memorial Sloan-Kettering Cancer Center by the DCC RSA. PPR is available Monday through Friday from 8:30am - 5:30 pm. Registrations must be faxed to the POETIC DCC and the Research Study Assistant (RSA) at MSKCC will verify eligibility and complete the registration with the PPR Office.

Once eligibility has been established and the participant is registered, the participant will be assigned an MSKCC Clinical Research Database (CRDB) number (protocol participant number). This number is unique to the participant and must be written on all data and correspondence for the participant. This protocol participant number will be relayed back to study staff at the registering site via e-mail and will serve as the enrollment confirmation.

The registration procedure is summarized below.

Confirm that the patient has received the Notice of Privacy Practice if local institutional requirement. This must be obtained before the eligibility confirmation and obtaining of the research informed consent.

Confirm eligibility as defined in the section entitled Criteria for Patient/Subject Eligibility.

Obtain written informed consent, by following procedures defined in Section 14.0, Recruitment Plan/ Informed Consent Procedures.

15.2 Randomization

This study does not include randomization.

16.0 DATA MANAGEMENT ISSUES

16.1 Data and Coordinating Center (DCC)

The Research Study Assistant (RSA) at POETIC DCC will be assigned to the study. The responsibilities of the RSA include project compliance, data collection, abstraction and entry, data reporting, regulatory monitoring, problems and prioritization. The Clinical Research Coordinator, CRS, is responsible for coordination between the RSA and Research Staff at the following member institutions: Alberta Children's Hospital, Memorial Sloan-Kettering Cancer Center, Phoenix Children's Hospital, Children's Hospital of Colorado, Children's Mercy Hospital and Clinics, and Pennsylvania State University College of Medicine, is necessary in order for a complete process. The CRS

at the POETIC DCC will also serve as the liaison among all staff involved including the principal investigators, attending physicians, and nurses.

Case report forms will be drafted in a standard format and will be provided to each participating institution by the DCC. The participating Site PI is responsible for ensuring these forms are completed accurately and legibly. The Site PI is also responsible for ensuring that all CRFs and corresponding source documentation is submitted within one week of the end of a treatment cycle. Required study tools for each protocol including correlative studies, vital signs, drug administration and protocol evaluations will also be provided to each participating institution by the DCC. Case report forms, study tools and source documentation are required to be submitted to the DCC one week after the completion of each cycle. The data collected for this study will be entered into a secure database by the RSA at the POETIC DCC. Data will be collected, stored, and monitored at an institutional level via the Clinical Research Database (CRDB) system. Data will be provided from CRDB to protocol-defined sponsors (CTEP, FDA, etc.) as required, through the Data Management Resource Division, a division of the Office of Clinical Research.

Source documentation will be available to support the computerized patient record and must be submitted with the case report forms and required study tools. Case report forms will not be considered source documentation.

- Variables that will be recorded include the patient's birth date, date of diagnosis, date of study entry and histologic diagnosis.
- The results of the pretreatment and end of therapy evaluations, including the extent of disease evaluation (history, physical examination and imaging studies), baseline laboratory values, renal and hepatic function, as defined per protocol, will be recorded.
- All study related treatment data and concomitant drugs will be recorded.
- The presence of toxicity at baseline, during and for 30 days after administration of the investigational agent will be monitored and recorded.
- The results of the extent of disease evaluation (history, physical examination and imaging studies) following each course of treatment will be recorded.
- The patient's disease status and last follow-up will be recorded. If disease progresses or recurs, the results of the repeat extent of disease evaluation will be recorded.

16.2 Site Research Staff

Research staff will be assigned at Alberta Children's Hospital, Memorial Sloan-Kettering Cancer Center, Phoenix Children's Hospital, Children's Hospital of Colorado, Children's Mercy Hospital and Clinics, and Pennsylvania State University College of Medicine. Their responsibilities will include project compliance, data collection, abstraction and entry, data reporting, regulatory monitoring, problems and prioritization, maintaining file documentation of data for the clinical trial, pharmacokinetic or other biologic correlative study collection, and analysis as outlined for each patient enrolled on study. They will also be responsible for maintaining a regulatory binder for each protocol. The designated research staff will also be responsible for submitting the data on a weekly basis by fax or mail to the RSA at the DCC. Case report forms and required study tools along with

supporting source documentation should be faxed/emailed or mailed to the address below, one week after the completion of each study cycle:

POETIC Data and Coordinating Center
Memorial Sloan Kettering Cancer Center
Pediatric & Allo Transplant Clinical Trials Office
405 Lexington Avenue, Room 3-512
New York, NY 10174
Phone: 646-888-5714/5715
Fax: 646-888-5726

16.3 Administrative Support

The protocol will be conducted as a single research study effort and data from each participating institution will be included in the analysis of results.

The Protocol Chairman will be responsible for the conduct of the study, monitoring of the progress of the study, and review of all case report forms from each participating institution.

Failure to submit required forms in the timelines requested will result in suspension of accrual privileges at a given site until data is updated, and/or withholding of contract payments if applicable.

Initial Protocol Submission

Prior to implementing the protocol, informed consent form, HIPAA authorization and any other information pertaining to participants must be approved by the MSKCC IRB/PB. Prior to implementing this protocol at the participating centers, approval must be obtained from the participating center's Local IRB of Record. The following documents must be provided to MSKCC before the participating site can be initiated and begin enrolling participants:

- Local IRB of Record approval(s) for the protocol, appendices, informed consent form and HIPAA authorization
- Local IRB approved consent form
- Local IRB of Record membership
- Local IRB of Record's Federal Wide Assurance number and OHRP Registration number
- Curriculum vitae and medical license for each investigator and consenting professional
- Documentation of Human Subject Research Certification and HIPAA training for investigators and key staff members
- Signed and dated FDA Related Forms 1572/1571 (if applicable)
- Lab Certifications and Reference Ranges for each lab listed on the 1572
- Appropriate financial disclosure forms.

Protocol Amendments/Status Changes

Each change to the protocol must be organized and documented by the POETIC DCC. After IRB approval at the lead institution, the POETIC DCC will distribute the amendment to the participating institutions, for approval by their local IRB within 60 days of the

amendment date. The participating sites will ensure that documentation for all IRB approved amendments are sent to the DCC and are maintained in the regulatory binder. This documentation will include the IRB approval letter referencing the protocol version date and amendment number, IRB approved protocol, IRB approved appendices and IRB approved consent forms.

The amendment will be written so that no other institution will need to reformat the information but can simply copy and distribute. An amendment memo as well as highlighted and clean copies of the protocol, appendices and consent forms will be distributed to the participating sites. The consent form will be a sample which may be edited in order to adhere to local IRB guidelines. The amendment must be submitted at each site to the IRB for review and approval before patients can be enrolled on the study and within 60 days of the amendment version date. The amendment number and version date will also be displayed on each amendment.

16.4 Additional IRB Correspondence

Annual re-approval

Annual continuing review reports from a participating center's Local IRB of Record must be submitted to MSKCC at the time re-approval is granted. The most current approved version of the consent form should also be submitted to MSKCC at that time. Failure to submit the reapproval will result in suspension of accrual privileges.

Deviations and Violations

If a deviation from the protocol is proposed for a potential or existing participant at a participating site, approval from the MSKCC IRB/PB is required prior to the action.
For all protocol violations, the participating site should report the violation to Dr. Tanya Trippett the Director of the POETIC DCC, as soon as possible. Dr. Trippett will in turn the report of the violation to the MSKCC IRB/PB. Participating sites should report deviations and violations to the Local IRB of Record as they occur. Approvals/acknowledgments from the Local IRB of Record for protocol deviations and violations should be submitted to POETIC DCC as received.

Other correspondence

Participating sites should submit other correspondence to their local IRB of Record according to local guidelines, and submit copies of that correspondence to MSKCC.

16.5 Quality Assurance

Quality assurance is a central responsibility of the POETIC DCC and it will be achieved by frequent review, constant oversight and input from objective advisors. Each project will maintain a steering committee which will meet in conference call on a weekly basis to critically review the scientific progress of the clinical trial. The steering committee will include the principal investigators, research staff and the biostatistical support team under the direction of Dr. Irina Ostrovnaya. Conference calls will also occur bi-weekly between the DCC and the Protocol Chair to discuss the trial.

Case Report Forms with accompanying source documents must be provided to the DCC to ensure that real-time monitoring can be accomplished. Queries will be generated by the DCC as needed if questions arise, and prompt responses are requested back to the

DCC. Internal audits will be conducted by the DCC. Audits may be accomplished in one of two ways: (1) source documents and research records for selected patients are brought from participating sites to the DCC for audit, or (2) selected patient records may be audited on-site at participating sites. These audits will be performed by persons who are qualified by training and experience to monitor the progress of the investigation. During these audits the following activities will take place:

- Review regulatory binders for protocol documentation;
- Ensure that case report forms are source data verified according to the monitoring plan
- Verify drug accountability is complete and accurate; and
- Verify compliance to GCPs, ICH guidelines, FDA regulations, and applicable SOPs.

If the sponsor chooses to have an audit at the DCC, then the DCC is responsible for having all source documents, research records, all IRB approval documents, Drug Accountability Record Forms, patient registration lists, response assessments scans, x-rays, etc. available for the audit. Alternatively, an external auditor would be designated for this study through a contract with MSKCC.

16.6 Data and Safety Monitoring

The Data and Safety Monitoring Committee (DSMC) under the direction of Dr. Robert Motzer and Bonnie Edelman, Manager, will be responsible for monitoring the data safety of the protocols sponsored by POETIC. The DSMC meets quarterly, and will review data from phase I, II, I/II, pilot and non-phase trials that are not being monitored by an industrial sponsor at least every 6 weeks, and which meet the NCI definition of a Clinical Trial.. A copy of the MSKCC Data and Safety Monitoring Plan is on file at the Data and Coordinating Center. The DSMC reports to MSKCC Research Council and Institutional Review Board.

The Data and Safety Monitoring (DSM) Plans at Memorial Sloan-Kettering Cancer Center were approved by the National Cancer Institute in September 2001. The DSM Plans at MSKCC were established and are monitored by the Office of Clinical Research. The plans address the new policies set forth by the NCI in the document entitled “Policy of the National Cancer Institute for Data and Safety Monitoring of Clinical Trials” which can be found at: http://cancertrials.nci.nih.gov/clinicaltrials/conducting/dsm-guidelines.

During the protocol development and review process, each protocol will be assessed for its level of risk and degree of monitoring required. Risk levels are as follows:

Low Risk: Probability of harm or discomfort not greater than daily life or routine physical/psychological exams.

Moderate Risk: Intervention commensurate with those inherent in their expected medical, social, or education situations. Risks are reasonable in relation to anticipated benefits and the importance of knowledge expected to result.

High Risk: Pose greater than minimal risk which may or may not have direct benefit to the participant.

High risk trials are monitored once per quarter, moderate risk twice per year and low risk once annually.

16.7 Therapeutic Response Review

The Therapeutic Response Review Committee (TRRC) is MSKCC's independent response review committee which annually evaluates therapeutic responses for participants in IRB/PB approved clinical trials where therapeutic efficacy is a stated primary objective, typically phase II and III trials. The process, done in an unbiased blinded fashion, ensures the data from the institution's therapeutic trials clinical research program is verified and vetted. Studies monitored by the TRRC include NCI-NIH, in-house, industrial trials, and multi-center trials where MSKCC is the coordinating center, which are not reviewed by an outside independent therapeutic response review board.

MSKCC's TRRC review will serve as the committee that may review and confirm responses of POETIC patients. The TRRC will possibly randomly select cases on an annual basis. If a patient is selected to be reviewed by the TRRC, the clinical site will be notified by the DCC with the required materials to be submitted to the committee and timeframe of submission.

17.0 PROTECTION OF HUMAN SUBJECTS

17.1 Privacy

All institutional, FDA, and NCI requirements for human subjects must be met. This study will be carried out in compliance with the regulations of the Health Insurance Portability and Accountability Act (HIPAA). Each participating institution will have an appropriate assurance on file with the Office for Human Research Protection (OHRP), NIH. The Data and Coordinating Center is responsible for assuring that each participating institution has an OHRP assurance and must maintain copies of IRB approvals from each participating site. The Data and Coordinating Center is responsible for assuring that IRB approval has been obtained at each participating site prior to the first patient registration from that site.

The risks and benefits of participation in this study will be reviewed with the patient and/or parent/legal guardian.

Enrollment on this study is on a voluntary basis and every effort will be made to maintain privacy and confidentiality. The patient's records will be confidential. Only authorized individuals or agencies may inspect the records. No identifying information will be used in reports or publications resulting from this study.

<u>The following section is a Mandatory Section that pertains to MSKCC only</u>:

It is the responsibility of the Research Staff to ensure that protocol subjects received the Center's Notice of Privacy Practices. If the subject has not received one, MSK personnel must provide a Notice of Privacy Practices and obtain acknowledgment before the subject participates in the study.

MSKCC's Privacy Office may allow the use and disclosure of protected health information pursuant to a completed and signed Research Authorization form. The use and disclosure of protected health information will be limited to the individuals described in the Research Authorization form. A Research Authorization form must be completed by the Principal Investigator and approved by the IRB and Privacy Board.

REFERENCES/BIBLIOGRAPHY

1. Folkman J. What is the evidence that tumors are angiogenesis dependent? J Natl Cancer Inst. 1990; 82: 4-6. (a)

2. Mainou-Fowler T, Angus B, Miller S, Proctor SJ, Taylor P, Wood KM. Micro-vessel density and the expression of vascular endothelial growth factor (VEGF) and platelet-derived endothelial cell growth factor (PdEGF) in classical Hodgkin Lymphoma. Leukemia 2006; 47:223-230.

3. Poon RT, Fan ST, and Wong J. Clinical implications of circulating angiogenic factors in cancer patients. J Clin Oncol 2001:19:1207-1225.

4. Senger DR, Perruzzi CA, Feder J, and Dvorak HF. A highly conserved vascular permeability factor secreted by a variety of human and rodent tumor cell lines. Cancer Res 1986;46: 5629-32.

5. Senger Dr, Galli SJ, Dvorak AM, Pemuzzi CA, Harvey VS, and Dvorak HF. Tumor cells secrete a vascular permeability factor that promotes accumulation of ascites fluid. Science 1983;219:983-985.

6. Folkman J. Fundamental concepts of the process of angiogenesis. Current Mol Med 2003;3:643-651.

7. Leung DW, Cachianes G, Kuang WJ, Goeddel DV, Ferrara N. Vascular endothelial growth factor is a secreted angiogenic mitogen. Science1989; 246: 1306-1309.

8. Kaplan RN, Riba RD, Zacharoulis S, Bramley AH, Vincent L, Costa C, MacDonald DD, Jin DK, Shido K, Kerns SA, Zhu Z, Hicklin D, Wu Y, Port JL, Altorki N, Port ER, Ruggero D, Shmelkov SV, Jensen KK, Rafii S, Lyden D. VEGFR1-positive haematopoietic bone marrow progenitors initiate the pre-metastatic niche. Nature 2005; 438:820-927.

9. Kaya M, Wada T, Akastsuka T et al. Vascular endothelial growth factor expression in untreated osteosarcoma is predictive of pulmonary metastasis and poor prognosis. Clin Cancer Res. 2000; 6:572-577.

10. Langer,I. *et al.* Expression of vascular endothelial growth factor (VEGF) and VEGF receptors in human neuroblastomas. Med Pediatr Oncol. 2000; 34: 386-393.

11. Salven P, Orpana A, Teerenhori L, Joensuu H. Simultaneous elevation in the serum concentrations of the angiogenic growth factors VEGF and bFGF is an independent predictor of poor prognosis in non-Hodgkin lymphoma: a single-institution study of 200 patients. Blood 2000; 96: 3712-3718.

12. Arush MW, Barak AB, Maurice S, Livne E. Serum VEGF as a significant marker of treatment response in Hodgkin Lymphoma. Ped Hematol Oncol 2007; 24:111-115.

13. Kieran M Anti-angiogenic therapy in pediatric neuro-oncology. J Neurooncol. 2005; 75:327-34.

14. Hurwitz,H. *et al.* Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. N Engl J Med 2004; 350:2335-2342.

15. Rowe,D.H. *et al.* Suppression of primary tumor growth in a mouse model of human neuroblastoma. J Pediatr Surg 2000;35:977-981.

16. Ozcan C, Wong SJ, Hari P. Reversible posterior leukoencephalopathy syndrome and bevacizumab [letter]. N Engl J Med 2006; 354:981–2.

17. Glusker P, Recht L, Lane B. Reversible posterior leukoencephalopathy syndrome and bevacizumab [letter]. New Engl J Med 2006;354:980-1.

18. Hambleton J, Skillings JR, Kabbinavar F, et al. Safety of low-dose aspirin (ASA) in a pooled analysis of 3 randomized, controlled trials (RCTs) of bevacizumab (BV) with chemotherapy (CT) in patients (pts) with metastatic colorectal cancer (mCRC). J Clin Oncol 2005;23 Suppl 16S:3554.

19. Scappaticci FA, Fehrenbacher L, Cartwright T, et al. Surgical wound healing complications in metastatic colorectal cancer patients treated with bevacizumab. J Surg Oncol 2005;91:173–80.

20. Sandler A, Gray R, Perry MC, et al. Paclitaxel-carboplatin alone or with bevacizumab for non-small-cell lung cancer. New Engl J Med 2006;355(24):2542–50.

21. Miller KD, Chap LI, Holmes FA, et al. Randomized Phase III trial of capecitabine compared with bevacizumab plus capecitabine in patients with previously treated metastatic breast cancer. J Clin Oncol 2005; 23:792–9.

22. Karp JE, Gojo I, Pili R, Gocke CD, Greer J, Guo C, Qian D, Morris L, Tidwell M, Chen H, and Zweibel J. Targeting vascular endothelial growth factor for relapsed and refractory adult acute myelogenous leukemias: therapy with sequential 1-beta-d-arabinofuranosylcytosine, mitoxantrone, and bevacizumab. Clin Cancer Res. 2004; 10(11): 3577-85.

23. Wedam SB, Low JA, Yang X, et al. A pilot study to evaluate response and angiogenesis after treatment with bevacizumab in patients with inflammatory breast cancer. J Clin Oncol 2004; 22 Suppl 14S:578.

24. D'Adamo DR, Anderson S, Albritton K, et al. Cardiac toxicity in a phase II study of doxorubicin and bevacizumab for patients with metastatic soft-tissue sarcomas. J Clin Oncol 2004;22 Suppl 14S:9012.

25. Kretschmar CS, Kletzel M, Murray K, Thorner P, Joshi V, Marcus R, Smith EI, London WB, and Castleberry R. Response to paclitaxel, topotecan, and topotecan-cyclophosphamide in children with untreated disseminated neuroblastoma treated in an upfront phase II investigational window: a pediatric oncology group study. J Clin Oncol. 2004 Oct 15;22(20):4119-26.

26. Allen JC and Helson L. High-dose cyclophosphamide chemotherapy for recurrent CNS tumors in children. J Neurosurg 1981; 55(5):749-56.

27. Stewart CF, Iacono LC, Chintagumpala M, et al. Results of a phase II upfront window of pharmacokinetically guided topotecan in high-risk medulloblastoma and supratentorial primitive neuroectodermal tumor. J Clin Oncol 2004; 22(16):3357-365.

28. Kadota RP, Stewart CF, Horn M, et al. Topotecan for the treatment of recurrent or progressive central nervous system tumors - a pediatric oncology group phase II study. J Neurooncol 1999; 43(1):43-47.

29. Frangoul H, Ames MM, Mosher RB, et al. Phase I study of topotecan administered as a 21-day continous infusion in children with recurrent solid tumors: a report from the Children's Cancer Group. Clin Cancer Res 1999; 5(12):3956-3962.

30. Willett CG, Boucher Y, Duda DG, di Tomaso E, Munn LL, Tong RT, Kozin SV, Petit L, Jain RK, Chung DC, Sahani DV, Kalva SP, Cohen KS, Scadden DT, Fischman AJ, Clark JW, Ryan DP, Zhu AX, Blaszkowsky LS, Shellito PC, Mino-Kenudson M, Lauwers GY. Surrogate markers for antiangiogenic therapy and dose-limiting toxicities for bevacizumab with radiation and chemotherapy: continued experience of a phase I trial in rectal cancer patients. Journal of Clinical Oncology 2005; 23: 8136-8139.

31. Tong RT, Boucher Y, Kozin SV, Winkler F, Hicklin DJ, Jain RK. Vascular normalization by vascular endothelial growth factor receptor 2 blockade induces a pressure gradient across the vasculature and improves drug penetration in tumors. Cancer Research 2004; 64:3731-6.

32. Winkler F, Kozin SV, Tong RT, Chae SS, Booth MF, Garkavtsev I, Xu L, Hicklin DJ, Fukumura D, di Tomaso E, Munn LL, Jain RK. Kinetics of vascular normalization by VEGFR2 blockade governs brain tumor response to radiation: role of oxygenation, angiopoietin-1, and matrix metalloproteinases. Cancer Cell 2004; 6: 553-563.

33. Jain RK, Tong RT, Munn LL. Effect of vascular normalization by antiangiogenic therapy on interstitial hypertension, peritumor edema and lymphatic metastasis: insights from a mathematical model. Cancer Res. 2007; 67:2729-35.

34. Dickson PV, Hamner JB, Sims TL, Fraga CH, Ng CY, Rajasekeran S, Hagedorn NL et al. Bevacizumab-induced transient remodeling of the vasculature in neuroblastoma xenografts results in improved delivery and efficacy of systemically administered chemotherapy. Clin Cancer Res 2007; 13(13):3942-3950.

35. Arush MW, Barak AB, Maurice S, Livne E. Serum VEGF as a significant marker of treatment response in Hodgkin Lymphoma. Ped Hematol Oncol 2007; 24:111-115.

36. Condoleelis, J, Pollard JW. Macrophages: obligate partners for tumor cell migration, invasion, and metastasis. Cell. 2006;124:263-6.

37. Okamoto R, Ueno M, Yamada Y, Takahashi N, Sano H, Suda T, Takakura N. Hematopoietic cells regulate the angiogenic switch during tumorigenesis. Blood. 2005; 105: 2757-2763.

38. Lin EY, Li JF, Gnatovskiy L, Deng Y, Zhu L, Grzesik DA, Qian H, Xue XN, Pollard JW. Macrophages regulate the angiogenic switch in a mouse model of breast cancer. Cancer Research 2006; 66:11238-46.

39. Ruan J, Hyjek E, Kermani P, Christos PJ, Hooper AT, Coleman M, Hempstead B, Leonard JP, Chadburn A, Rafii S. Magnitude of stromal hemangiogenesis correlates with histologic subtype of non-Hodgkin's lymphoma. Clinical Cancer Research. 2006; 12: 5622-31.

40. Kaplan RN, Riba RD, Zacharoulis S, Bramley AH, Vincent L, Costa C, MacDonald DD, Jin DK, Shido K, Kerns SA, Zhu Z, Hicklin D, Wu Y, Port JL, Altorki N, Port ER, Ruggero D, Shmelkov SV, Jensen KK, Rafii S, Lyden D. VEGFR1-positive haematopoietic bone marrow progenitors initiate the pre-metastatic niche. Nature. 2005; 438:820-927.

41. Dome B, Timar J, Dobos J, Meszaros L, Raso, E, Paku S, Kenessey I, Ostoros G, Magyar M, Landanyi A, Bogos and Tovari J. Identification and Clinical Significance of Circulating Enothelial Progenitor Cells in Human Non-Small Cell Lung Cancer. Cancer Research. 2006; 66:7341-7347.

42. Fürstenberger G, von Moos R, Lucas R, Thürlimann B, Senn HJ, Hamacher J, Boneberg EM. Circulating endothelial cells and angiogenic serum factors during neoadjuvant chemotherapy of primary breast cancer. British Journal of Cancer. 2006; 94:524-31.

43. Beerepoot LV, Mehra N, Vermaat JS, Zonnenberg BA, Gebbink MF, Voest EE. Increased levels of viable circulating endothelial cells Annals of Oncology. 2004;15:139-145.

44. Lin EH, Hassan M, Li Y, Zhao H, Nooka A, Sorenson E, Xie K, Champlin R Wu, X, Li D. Elevated circulating endothelial progenitor marker CD133 messenger RNA levels predict colon cancer recurrence.

45. Igreja C, Courinha M, Cachaço AS, Pereira T, Cabeçadas J, da Silva MG, Dias S. Characterization and clinical relevance of circulating and biopsy-derived endothelial progenitor cells in lymphoma patients. Haematologica 2007;92:469-477.

46. Kosaka Y, Mimori K, Fukagawa T, Ishikawa K, Etoh T, Katai H, Sano T, Watanabe, M, Sasako, Mori M. Identification of the high-risk group for metastasis of gastric cancer cases by vascular endothelial growth factor receptor-1 overexpression in peripheral blood. British Journal of Cancer 2007; 96:1723-1728.

47. Drevs J, Zirrgiebel U, Schmidt-Gersbach CI, Mross K, Medinger M, Lee L, Pinheiro J, Wood J, Thomas AL, Unger C, Henry A, Steward WP, Laurent D, Lebwohl D, Dugan M, Marme D. Soluble markers for the assessment of biological activity with PTK787/ZK 222584 (PTK/ZK), a vascular endothelial growth factor receptor (VEGFR) tyrosine kinase inhibitor in patients with advanced colorectal cancer from two phase I trials. Ann Oncol. 2005; 16(4):558-65.

48. Shaked Y, Bocci G, Munoz R, Man S, Ebos JM, Hicklin DJ, Bertolini F, D'Amato R, Kerbel RS. Cellular and molecular surrogate markers to monitor targeted and non-targeted antiangiogenic drug activity and determine optimal biologic dose. Curr Cancer Drug Targets. 2005; 5(7):551-9.

49. Aref S, El Sherbiny M, Goda T, Fouda M, Al Askalany H, Abdalla D. Soluble VEGF/sFLt1 ratio is an independent predictor of AML patient out come. Hematology 2005; 10(2):131-4.

50. Klisch J, Kubalek R, Scheufler KM, Zirrgiebel U, Drevs J, Schumacher M. Plasma vascular endothelial growth factor and serum soluble angiopoietin receptor sTIE-2 in patients with dural arteriovenous fistulas: a pilot study. Neuroradiology 2005; 47(1):10-7.

51. Reusch P, Barleon B, Weindel K, Martiny-Baron G, Godde A, Siemeister G, Marme D. Identification of a soluble form of the angiopoietin receptor TIE-2 released from endothelial cells and present in human blood. Angiogenesis. 2001; 4(2):123-31.

52. Taylor AP, Osorio L, Craig R, Raleigh JA, Ying Z, Goldenberg DM, Blumenthal RD.Tumor-specific regulation of angiogenic growth factors and their receptors during recovery from cytotoxic therapy. Clin Cancer Res. 2002; 8(4):1213-22.

53. Cunningham D, Humblet Y, Siena S, et al. Cetuximab monotherapy and cetuximab plus irinotecan in irinotecan-refractory metastatic colorectal cancer. N Engl J Med. 2004;351:337-345.

54. Kurhanewicz J, Vigneron DB, Nelson SJ, al. e. Citrate as an in vivo marker to discriminate prostate cancer from benign prostatic hyperplasia and normal prostate peripheral zone: detection via localized proton spectroscopy. Urology. 1995;45:459-466.

55. Mackinnon WB, al. e. Fine-needle biopsy specimens of benign breast lesions distinguished from invasive cancer ex vivo with proton MR spectroscopy. Radiology. 1997;204:661-666.

56. Biomarkers Definition Working Group N. Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. Clinical Pharmacol Ther. 2001:89-95.

57. Schmidt C. Metabolomics takes its place as latest up-and-coming "omic" science. Journal of the National Cancer Institute. 2004;96:732-734.

58. Evelhoch JL, Gillies TJ, Karczmar GS, al. e. Applications of magnetic resonance in model systems: Cancer therapeutics. Neoplasia. 2000;2:152-165.

59. Griffiths JR, al. e. Metabolic changes detected by in vivo magnetic resonance studies of HEPA-1 wild-type tumor deficient in hypoxia-inducible factor 1-beta (HIF-1beta): evidence of an anabolic role for the HIF-1 pathway. Cancer Research. 2002;62:688-695.

60. Sterin M, Cohen JS, Mardor Y, Berman E, Ringel I. Levels of phospholipid metabolites in breast cancer cells treated with anti-mitotic drugs: a 31P-magnetic resonance spectroscopy study. Cancer Research. 2001;61:7536-7542.

61. Gottschalk S, Anderson N, Hainz C, Eckhardt SG, Serkova NJ. Imatinib (STI571)-mediated changes in glucose metabolism in human leukemia BCR-ABL positive cells. Clinical Cancer Research. 2004;10:6661-6668.

62. Ackerstaff E, Glunde K, Bhujwalla ZM. Choline phospholipid metabolism: A target in cancer cells ? J Cell Biol. 2003;90:525-533.

63. Baguley BC, Marshall ES. In vitro modelling of human tumour behaviour in drug discovery programmes. Eur J Cancer. 2004;40:794-801.

64. Agrawal S, Temsamani J, Tang JY. Pharmacokinetics, biodistribution, and stability of oligodeoxynucleotide phosphorothioates in mice. Proc Natl Acad Sci U S A. 1991;88:7595-7599.

65. Kaplan EL, Meier P. Nonparametric estimation from incomplete observations. J Am Stat Assoc. 1958;53:457-480.

66. Serkova N, Fuller TF, Klawitter J, Freise CE, Niemann CU. 1H-NMR-based metabolic signatures of mild and severe ischemia/re-perfusion injury in rat kidney transplants. Kidney Int. 2005;67:1142-1151.

67. Smith A, Hennessy J, Heisel Kurth M, and Nelson S. Reversible skeletal changes after treatment with bevacizumab in a child with cutaneovisceral angiomatosis with thrombocytopenic syndrome. Pediatr Blood Cancer2008; 51(3): 418-420.

68.Glade-Bender JL, Adamson PC, Reid JM, Xu L, Baruchel S, Shaked Y, Kerbel RS,,Cooney-Qualter EM, Stempak D, Chen HX, Nelson MD, Krailo MD, Ingle AM, Blaney SM, Kandel JJ, Yamashiro DJ. Phase I trial and pharmacokinetic study of bevacizumab in pediatric patients with refractory solid tumors: A Children's Oncology Group Study. J Clin Oncol 2008, 26(3): 399-405.

69. Saylors RL, Stine KC, Sullivan J, Kepner JL, Wall DA, Bernstein ML, Harris MB, Hayashi R, Vietti TJ. Cyclophosphamide plus topotecan in children with recurrent or refractory solid tumor: A Pediatric Oncology Phase II Study. J Clin Oncol 2001, 19:3463-3469.

70. Griffin, JL, Shockcor JP. Metabolic profiles of cancer cells. Nat Rev Cancer. 2004 Jul:4(7):551-61.

71. Figure provided by Dr. David Lyden on page 76 of the Appendix (Figure 1).

Figure 1.

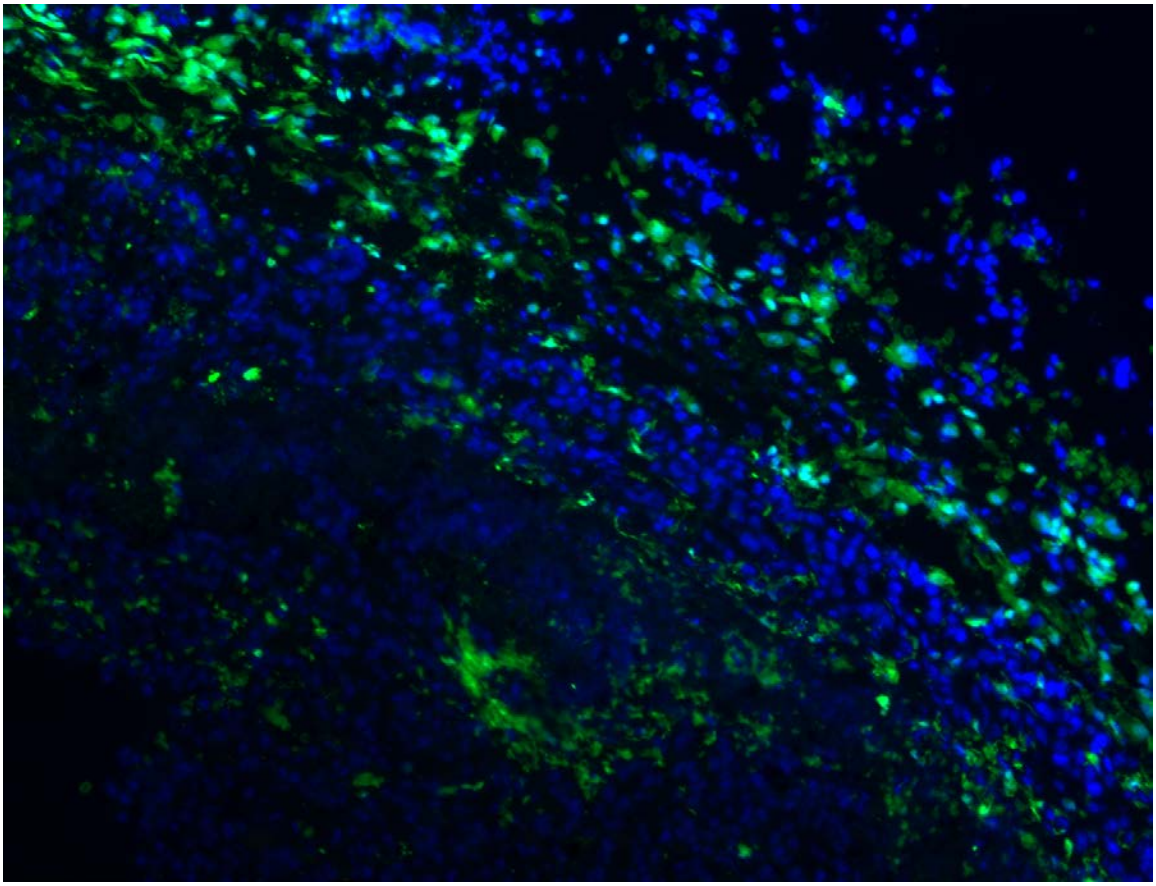
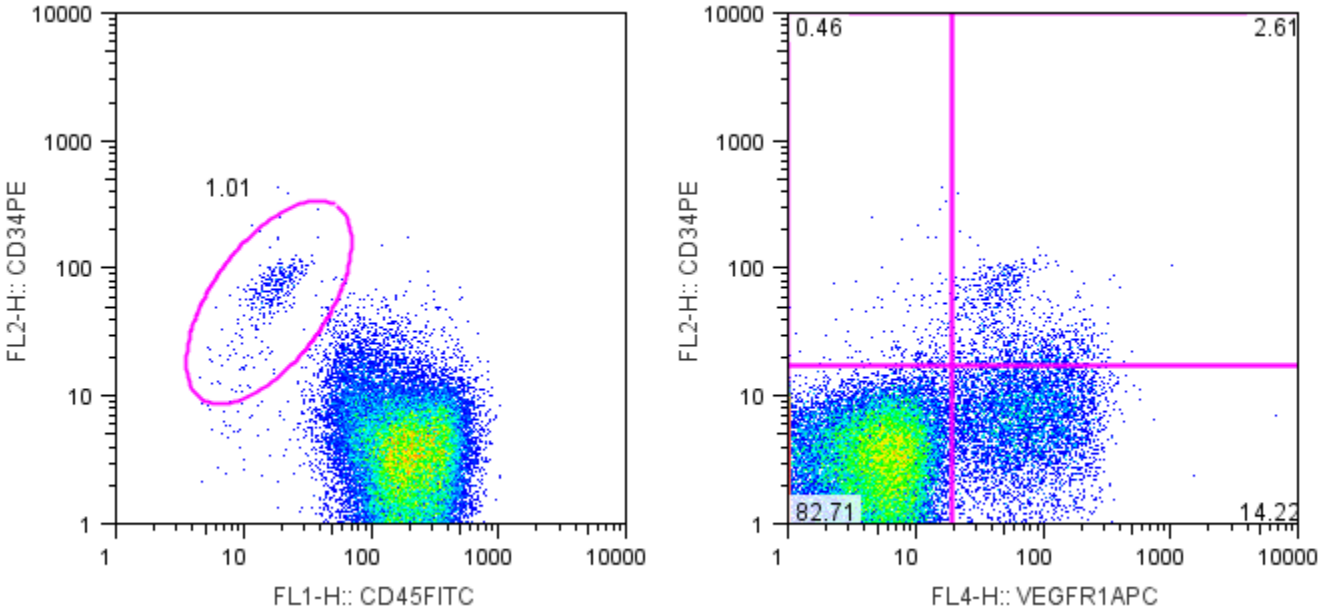


Figure 1: Peripheral rim of tumor with blue Dapi staining nuclei and green fluorescent protein expressing cells are bone marrow-derived and localize to the invasive front of the tumor.

Figure 2.

Normal Patient Control mononuclear cells stained for CD45, CD34 and VEGFR1



Breast Cancer Patient mononuclear cells stained for CD45, CD34 and VEGFR1

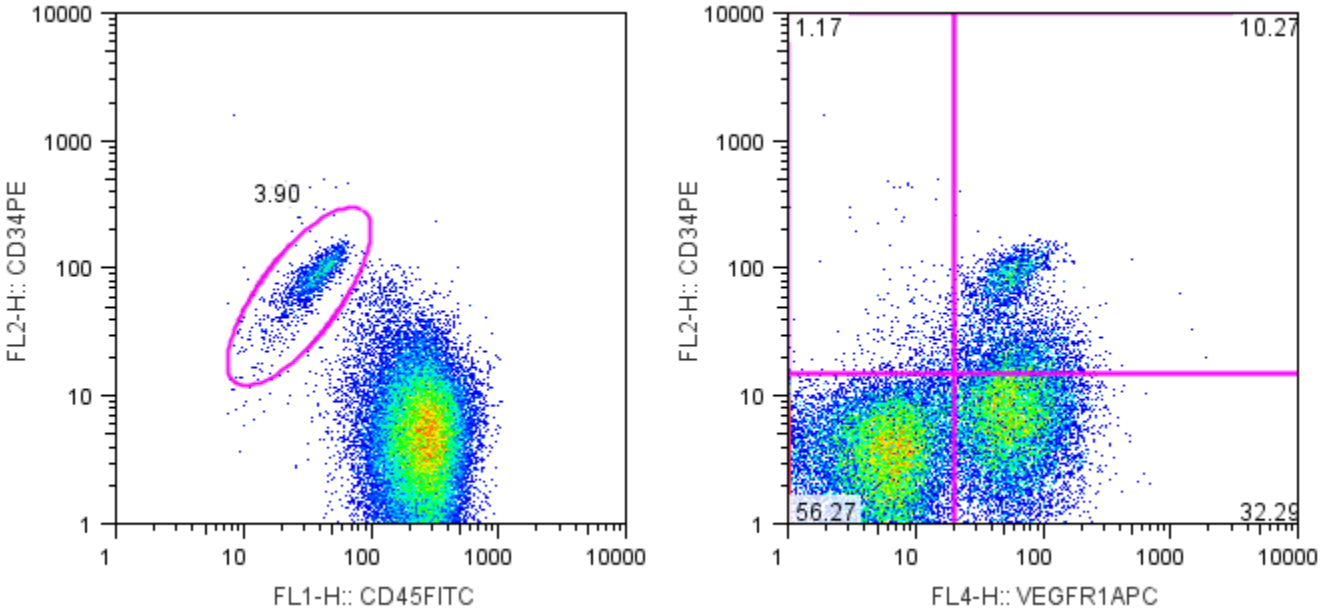


Table 3

	Normal Controls (n=6)	Std. Dev.	New Diagnosis (n=4)	Std. Dev.	Remission (n=6)	Std. Dev.
$CD34^+/VEGFR1^-$ HSC/HPCs	0.41	0.33	0.46	0.37	0.368	0.368
CD34+/VEGFR1+ HPCs	**1.205**	0.713	**2.77**	0.869	**0.79**	0.824
$CD34^-/VEGFR1^+$ Mature monocytes	19.191	6.182	20.446	7.992	42.6	17.865
$CD133^+/VEGFR2^+$ EPCs	0.28	0.14	0.83	0.45	0.46	0.38

Table3: Levels of $CD34^+/VEGFR1^-$ HSC/HPCs, $CD34^+/VEGFR1^+$ HPCs, and $CD34^-/VEGFR1^+$ macrophages were measured in Normal Controls (n=6), patients with a new diagnosis of rhabdomyosarcoma (n=4) and in remission (n=6). Patients with new diagnosis had the highest HPCs and there was a reduction in these levels in patients who were with no evidence of disease after completion of therapy.