Combination Of Bevacizumab, Irinotecan And Temozolomide For Relapsed Or Refractory Neuroblastoma: A Phase II Study

MSKCC THERAPEUTIC/DIAGNOSTIC PROTOCOL

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Please Note: A Consenting Professional must have completed the mandatory Human Subjects Education and Certification Program.

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1.0 PROTOCOL SUMMARY AND/OR SCHEMA

Neuroblastoma (NB) remains a fatal disease for a large percentage of patients, especially those with high-risk features including those that relapse or are resistant to conventional therapy. The combination of irinotecan and temozolomide is widely used as salvage chemotherapy for resistant NB both at MSKCC and nationwide. Although patients often have stabilization of their disease, response rates are relatively low and responses are usually not durable. The anti-vascular endothelial growth factor (VEGF) monoclonal antibody bevacizumab has been studied in children in a phase I study without dose-limiting toxicities (DLT) being encountered. In preclinical studies, bevacizumab enhanced irinotecan-induced suppression of NB xenografts. In this phase II study, we will evaluate effectiveness of the combination of intravenous (IV) bevacizumab, IV irinotecan and oral temozolomide. Patients will be monitored for toxicity and changes in angiogenic profile will be assessed as a secondary objective. The overall schema for each cycle of chemotherapy is shown below in Table 1.

Table 1: Schema for therapy for each cycle

Day	Intervention
1	Bevacizumab 15mg/kg IV
4-8	Irinotecan 50mg/m^2/day+temozolomide 150mg/m^2/day
15	Bevacizumab 15mg/kg IV

If eligible for second cycle, above schema will be repeated starting between days 29-43 (Section 9.8). Extent of disease evaluation will be carried out on day 15-35 of **second** cycle. If there is no progressive disease (PD) and the patient is found to be eligible for further treatment, the above schema will be repeated. Extent of disease evaluation will be performed after every two cycles. If patients continue to be eligible for therapy and do not have progressive disease, they may continue to receive therapy with bevacizumab, irinotecan and temozolomide for a maximum of two years.

2.0 OBJECTIVES AND SCIENTIFIC AIMS

2.1 Primary Objectives

- To evaluate tumor responses to combination of irinotecan, temozolomide and bevacizumab in patients with resistant NB.

2.2 Secondary Objectives

- To determine the toxicity of the combination of irinotecan, temozolomide and bevacizumab in patients with resistant NB.
- To evaluate changes in angiogenic markers after treatment with the combination of irinotecan, temozolomide and bevacizumab.
- To measure time to progression in patients with resistant NB treated with the combination of irinotecan, temozolomide and bevacizumab.

3.0 BACKGROUND AND RATIONALE

3.1 Disease Background

NB, the most common extracranial solid tumor of childhood accounts for 8% of pediatric malignancies. 50-60% of patients present with an unresectable primary tumor and metastases in bone marrow (BM). Intensive induction chemotherapy and aggressive surgery have improved remission rates in young patients. However, results have been less impressive in adolescents and adults in whom NB is especially resistant[1-3]. Myeloablative therapy (with stem-cell support) has been the most common approach for eradicating minimal residual disease in distant sites. Although the vitamin A derivative 13-*cis*-retinoic acid (isotretinoin) helped to prolong relapse-free survival, the long-term relapse-free survival rate in the most recent national study was projected to be only ~20%[4, 5]. Furthermore, the ability to cure patients who relapse remains extremely low (<10%)[6, 7]. These results are compelling reasons for pursuing novel therapeutic approaches.

3.2 Approaches to salvage therapy for resistant neuroblastoma

Until recently the most common salvage regimen used for patients with relapsed and refractory disease was the combination of cyclophosphamide and topotecan[8, 9]. However, the Children's Oncology Group, the only cooperative pediatric oncology group in the US, recently introduced this combination as an upfront "window" treatment for newly diagnosed patients, making it a less attractive proposition for use in a relapse/refractory setting for fear of chemoresistance. Current second line therapies include anti-GD2 immunotherapy for patients who have BM but no skeletal disease[10], targeted endoradiotherapy (^{131}I-MIBG or ^{131}I-3F8)[11 12] or phase I/II agents. However, these therapies have considerable limitations including poor response rates, short lived responses and in the case of targeted radiotherapy, the need for availability of stored autologous hematopoietic stem cells[11] . Currently, the most widely used and well studied second line regimen for patients with refractory or relapsed NB is the combination of irinotecan and temozolomide[13, 14].

3.3 Irinotecan therapy of NB

Irinotecan is a camptothecin prodrug activated by carboxylesterases to the active topoisomerase I inhibitor SN-38. Irinotecan was shown to have anti-NB in xenograft models[15, 16]. It subsequently underwent phase I and phase II testing in children with NB using various dosing schedules.[17-19] Toxicities included diarrhea (early and delayed), relatively modest myelosuppression, nausea, vomiting and asthenia. In general, although stabilization of disease was often observed, response rates were <5%[20, 21]. At MSKCC, five-day courses of irinotecan alone were shown to be effective palliative therapy for patients (n=44) with resistant NB[22]

3.4 Temozolomide therapy of NB

Temozolomide, a methylating agent that is thought to act by generating O^6-methylguanine in DNA has been shown to have activity against some NB xenografts[23]. In a phase II study in patients with NB, 5/23 patients showed objective responses[24]. In a further study for several

pediatric solid tumors, 2/16 patients with NB had objective responses[25]. Myelosuppression was the main toxicity encountered.

3.5 Combination of irinotecan and temozolomide for NB

Methylation of O^6-guanine by temozolomide may lead to recruitment of topoisomerase I and potentially enhance the probability of inducing camptothecin-mediated damage. This forms the biochemical rationale for combining temozolomide with a camptothecin[26]. Furthermore the lack of overlapping major toxicities makes this an attractive combination for clinical use. A phase I study of the combination of temozolomide plus protracted irinotecan established toxicity profile in heavily pretreated patients. Toxicities included diarrhea and myelosuppression[27]. At MSKCC, we have treated thus far >80 patients with refractory or relapsed NB with irinotecan (50mg/m^2/day x 5 days administered as a 1hr infusion) plus temozolomide (150mg/m^2/day orally x 5 days). We reported on results on 49 patients who received 1 to 15 courses (median, 5)[13]. Gastrointestinal and myelosuppressive toxicities were readily managed. 2/19 patients treated for refractory NB and assessable for response had complete response (CR) and 1/17 patients treated for progressive disease showed partial response (PR). A further 9 patients had objective responses. Multiple courses entailed no cumulative toxicity and controlled disease for prolonged periods in many patients, including some who were unable to complete prior treatments because of hematologic, infectious, cardiac, or renal problems. Myelosuppression was manageable: in patients with pretreatment platelet counts more than 100,000/µL, courses could be started every 3 weeks. In patients with pretreatment platelet counts of 32,000 to 85,000/µL, courses could be started every 3 to 5 weeks.

3.6 Tumor angiogenesis in NB

Recent studies have implicated angiogenesis as a significant regulator of NB growth. In a study of 50 untreated patients, tumor vascularity correlated with presence of metastases at diagnosis, MYCN amplification, unfavorable histology and poor survival probability[28]. Advanced stage tumors have an increased numbers of microvessels[29, 30]and a significant association between high levels of expression of the integrins $\alpha_v\beta_3$ and $\alpha_v\beta_5$, which are markers of active angiogenesis[31]. Expression of the pro-angiogenic vascular endothelial growth factor (VEGF) and its receptor (VEGFR) has been demonstrated in NB cell lines and in primary tumor samples [32]. The coexpression of VEGF and VEGFR suggests the presence of an autocrine loop [32]. High levels of pro-angiogenic factors, including VEGF, VEGF-B, VEGF-C, basic fibroblast growth factor (bFGF), angiopoietin-2, transforming growth factor-α (TGF-α), and platelet derived growth factor-A (PDGF-A) were found in advanced-stage NB tumors. In addition, high expression of PDGF-A was significantly associated with decreased survival and has been associated with advanced stage in NB[33, 34].The hypoxia inducible factors (HIF) may also play a key role in the angiogenic process[35]. NB cells and stroma secrete matrix metalloproteinases MMP-2 and MMP-9 that degrade matrix and facilitate invasion and metastasis[36-38]. Because *MYCN* amplification is strongly associated with rapidly progressive malignant growth, many investigators have speculated that the *MYCN* oncogene may stimulate angiogenesis in NB by modulating the expression pattern of angiogenesis factors[39]. In support of this hypothesis, down-regulation of Activin A, a developmentally regulated anti-angiogenic TGF-family member has been observed in supernatants of SH-EP NB cells with exogenous *MYCN* expression[40]. Recently, interleukin-6

(IL-6) has also been shown to have anti-angiogenic activity and to be down-regulated in *MYCN*-transfected NB cells[41]. In contrast to high-risk NB, low risk-NB frequently have a stroma-rich component made up of mitotically quiescent Schwann cells. Schwann cells have been shown to produce the anti-angiogenic agents tissue inhibitors of metalloproteinase (TIMPs) and secreted protein acidic and rich in cysteine (SPARC) that may contribute to the relatively avascular nature of most stroma-rich low-risk NB[42, 43].

3.7 Anti-VEGF therapy for NB

VEGF is considered to play a critical role in the initiation of tumor angiogenesis[44, 45]. In NB, expression of VEGF and VEGFR have been correlated by some investigators with higher stage NB[46, 47] . Furthermore, VEGF may act as a growth factor for NB cells independent of its pro-angiogenic action[32, 48]. Multiple anti-VEGF strategies including small molecule inhibitors such as tyrosine kinase inhibitors[49, 50], decoy receptor[51], methionine aminopeptidase-2 inhibitors (e.g. TNP470)[52], cytokines[53], chemotherapeutic agents[54, 55] and antibodies have been used in preclinical NB models. In general, apart from conventional chemotherapeutic agents that have multiple mechanisms of action, these have at best only partially suppressed growth of established NB xenografts [49, 51, 56-58]. Similarly in studies carried out in our laboratory (see Section 3.11), the anti-VEGFR antibody bevacizumab did not significantly suppress established NB xenografts. Though some preclinical studies suggest that bevacizumab may have some modest anti-NB activity in the minimal disease setting[59], NB may have other mechanisms of sustaining blood supply including upregulation of proangiogenic factors[60]. This suggests that anti-VEGF therapy is best used in combination with other anti-NB agents.

3.8 Bevacizumab: preclinical studies

Bevacizumab is a humanized monoclonal antibody[61] binding all five isoforms of human VEGF that currently has FDA approval for the therapy of metastatic colorectal carcinoma and lung carcinoma. Its binding epitope[62] and pharmacokinetic properties[63] have been described. It has anti-tumor activities in murine xenograft models[61] and its safety has been studied in cynomolgus monkeys[64]. Significant preclinical toxicities observed were as follows. (a) Physeal dysplasia of the distal femur: these were zones of bone growth cessation in animals with open growth plates. These were dose-related and were reversible on cessation of bevacizumab. (b) Decrease in ovarian and uterine weights were observed in female monkeys along with absence of corpora lutea. These were also reversible on cessation of the agent[61]. (c) Wound healing: dermal wound healing was found to be impaired in a rabbit model[65]. (d) Proteinuria in mice: this was observed within 3 hours after IV administration and was associated with glomerular hypertrophy, endothelial detachment from glomerular basement membrane and disruption of slit diaphragms. This correlated with the decreased expression of glomerular slit diaphragm/podocyte-associated protein nephrin[66].

3.9 Bevacizumab: clinical studies in adults

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.

In a large phase III study (AVF2107g) [67] 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. 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. 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 non-small cell lung cancer (NSCLC) in October 2006[68].

3.10 Bevacizumab: safety profile

Common adverse events reported with the use of bevacizumab included reactions typically encountered with monoclonal antibody administration and included headache, fever, fatigue, nausea, vomiting, arthralgia and rash. 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), 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.

3.10.1 Hypertension: Hypertension has been commonly seen in bevacizumab clinical trials to date and oral medications have been used to manage the hypertension when indicated. In two phase III studies, the incidence of hypertension (and of grade 3 hypertension) was reported to be increased[67, 69] 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) [70, 71]. RPLS may include signs and symptoms of headache, altered mental function, seizures, and visual disturbances / cortical blindness and requires treatment, which should include control of hypertension, management of specific symptoms, and permanent discontinuation of bevacizumab. 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.

3.10.2 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.

3.10.3 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. In the phase III pivotal trial in metastatic CRC, there was a slightly higher rate of venous TE events that was not statistically significant in patients treated with bevacizumab plus chemotherapy compared with chemotherapy alone (19% vs. 16%). The incidence of NCI-CTC Grade ≥ 3 venous TE (VTE) events in one NSCLC trial (ECOG4599) 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; non- fatal events were reported in the carboplatin/paclitaxel arm (see Bevacizumab Investigator Brochure). 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.

There was also a higher rate of ATE event (3% vs. 1%) such as myocardial infarction, transient ischemia attack, cerebrovascular accident/stroke and angina/unstable angina. A pooled analysis of the rate of ATE events from 5 randomized studies (1745 patients) showed that treatment with chemotherapy plus bevacizumab increased the risk of having an ATE event compared with chemotherapy alone (3.8% vs. 1.7%, respectively) [72]. 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. Furthermore, subjects with certain baseline characteristics (age ≥ 65 years and/or a history of a prior ATE event) may be at higher risk of experiencing such an event. [69, 73]. See the bevacizumab Investigator Brochure for additional information on risk factors.

Aspirin is a standard therapy for primary and secondary prophylaxis of ATE 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[74]. Further analyses of the effects of concomitant use of bevacizumab and aspirin in colorectal and other tumor types are ongoing.

3.10.4 Gastrointestinal (GI) perforation Patients with metastatic carcinoma may be at increased risk for the development of GI perforation when treated with bevacizumab and chemotherapy. Bevacizumab should be permanently discontinued in patients who develop gastrointestinal perforation. A causal association of intra-abdominal inflammatory process and gastrointestinal perforation to bevacizumab has not been established. Nevertheless, caution should be exercised when treating patients with intra-abdominal inflammatory processes with bevacizumab. GI perforation has been reported in other trials in non-colorectal cancer populations (e.g., ovarian, renal cell, pancreas, and breast) and may be higher in incidence in some tumor types. GI perforation was not correlated to duration of exposure to bevacizumab[67].

3.10.5 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.

3.10.6 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[65] (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 20 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).

3.10.7 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 five of these cases, these hemorrhages were preceded by cavitation and/or necrosis of the tumor[75]. Tumor-associated hemorrhage was also seen rarely in other tumor types and locations, including central nervous system (CNS) bleeding in a patient with hepatoma with occult CNS metastases and continuous oozing of blood from a thigh sarcoma with necrosis. Serious GI hemorrhage has been reported in a phase II study for patients with colorectal cancer[73].

Across all bevacizumab clinical trials, mucocutaneous hemorrhage has been seen in 20%-40% of patients treated with bevacizumab. These were most commonly 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. Grade 1 epistaxis occurred in 32% patients receiving bevacizumab plus combination chemotherapy as first line therapy for metastatic colon cancer[69].

3.10.8 CHF: 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.[76] Two additional studies investigated concurrent administration of anthracyclines and bevacizumab. In 21 patients with inflammatory breast cancer treated with neoadjuvant docetaxel, doxorubicin, and bevacizumab, no patients developed clinically apparent CHF; however, patients had asymptomatic decreases in LVEF to < 40% [77]. In a small Phase II study in patients with soft tissue sarcoma, 2 of the 17 patients treated with bevacizumab and high-dose doxorubicin (75 mg/m^2) developed CHF (one Grade 3 event after a cumulative doxorubicin dose of 591 mg/m^2, one Grade 4 event after a cumulative doxorubicin dose of 420 mg/m^2); an additional 4 patients had asymptomatic decreases in LVEF [78]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.

3.10.9 Neutropenia: When combined with chemotherapy, bevacizumab increased the risk of neutropenia compared to chemotherapy alone. In a phase 3 trial with IFL +/- bevacizumab in colorectal cancer, grade 3-4 neutropenia was 21% in the bevacizumab + IFL arm vs. 14% in the IFL arm (grade 4 neutropenia was 3% vs. 2%). In a phase 3 trial with carboplatin and paclitaxel +/- bevacizumab in NSCLC, the bevacizumab containing arm was associated with an increased rate of grade 4 neutropenia (27% vs 17%), febrile neutropenia (5.4% vs. 1.8%), and an increased rate of infection with neutropenia (4.4% vs. 2.0%) with three fatal cases in the bevacizumab + chemotherapy arm vs. none in the chemotherapy control arm.

3.10.10 RPLS: 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[70, 71]

Additional Adverse Events: are described in bevacizumab Investigator Brochure

3.11 Pediatric experience with bevacizumab

Pediatric experience with bevacizumab is limited. In the only published study, the Children's Children's Oncology Group recently reported on a phase I study of bevacizumab in 20 patients with resistant solid tumors (including 2 patients with NB). In this study, patients received bevacizumab as a single agent administered IV every two weeks followed by a two-week break before repeat administration. 18 patients were evaluable for toxicity. The maximum planned dose of 15mg/kg/dose was achieved without DLT being encountered. Non-DLTs included infusional reaction, rash, mucositis, proteinuria, mild hypertension and lymphopenia. There was no hemorrhage or thrombosis. The median clearance of BV was 4.1 mL/d/kg (range, 3.1 to 15.5 mL/d/kg), and the median half-life was 11.8 days (range, 4.4 to 14.6 days). No objective responses were observed[79]. Other adverse events described in patients treated off study include RPLS[80] and reversible skeletal changes[81]. At MSKCC we have treated 16 patients thus far on a phase I study (MSKCC IRB 06-072) of radioiodinated 3F8 plus bevacizumab in which the latter is administered at 15mg/kg/dose. We have encountered one grade 3 DLT: a patient developed GI perforation. However, skeletal changes have not been observed[82] and in general, patients have tolerated therapy well.

3.12 Rationale for combining anti-angiogenic therapy with chemotherapy

Randomized control studies have confirmed the benefit of combining bevacizumab with cytotoxic therapy although the mechanism remains unclear. One hypothesis states that cytotoxic therapy releases VEGF which is then removed by bevacizumab leading to endothelial cell apoptosis and enhanced cancer cell death. Another proposes that bevacizumab selectively inhibits angiogenesis and "normalizes" intratumoral vasculature leading to a paradoxical improvement in perfusion and enhanced delivery of cytotoxic agents[83]. Both mechanisms may be involved in the anti-cancer effect of angiogenics. In our protocol, the first dose of bevacizumab injected prior to chemotherapy will avail of the latter hypothesis. It could be hypothesized that the second dose of bevacizumab injected 7 days after the last administration of chemotherapy may then remove VEGF released by the chemotherapeutic agents.

3.13 Rationale for combining irinotecan and temozolomide with bevacizumab for NB

In our laboratory we have recently observed bevacizumab-mediated enhancement of the anti-tumor effect of irinotecan in NB bearing xenografts. NB xenografts using 4 cell lines (LAN-1, NMB7, SKNBE(2)C and SKNLP) were established in athymic nude mice by injecting 2-5 x 10^6 cells subcutaneously in the flank. After tumor reached a diameter of 0.7-1cm, mice in groups of

4or 5 were either (a) left untreated, (b) treated with either irinotecan (0.75mg/kg IV daily Mon-Fri x 10 doses), (c) bevacizumab (5mg/kg IV twice weekly x 6 doses) alone or (d) combination of irinotecan and bevacizumab. Tumors were measured three times weekly and mice were sacrificed when tumors reached a diameter of 2cm. Tumor sizes were compared between groups using student t-test. Tumor growth curves are shown in Figure 1 below. Bevacizumab significantly enhanced the anti-tumor effect of irinotecan in all xenograft models: p values for comparison between irinotecan and irinotecan+bevacizumab groups were 0.04 for NMB7 on day 24, 0.005 for SKNLP on day 27, 0.05 on day 13 for LAN1.Tumor suppression resulted in a significantly enhanced survival in mice treated with the combination of bevacizumab and irinotecan when compared to control groups.

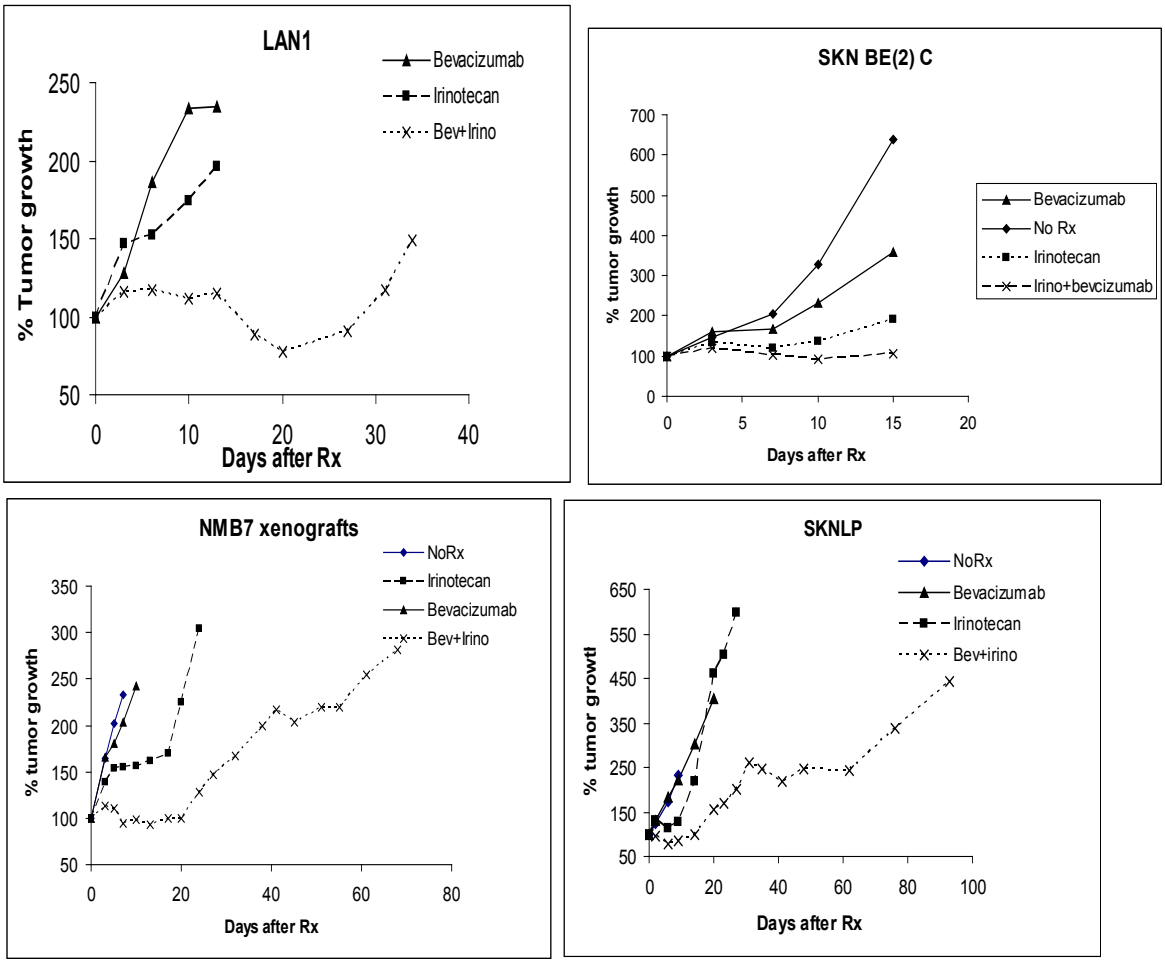

Figure 1. Bevacizumab enhances anti-NB effect of irinotecan in NB xenograft mouse models

Given the enhanced anti-NB activity of the combination of bevacizumab+ irinotecan compared to irinotecan alone, we propose to add bevacizumab to the established salvage regimen for NB and propose to study the combination of bevacizumab+irinotecan+temozolomide in patients with relapsed or refractory NB. This will be the first study of this combination in a predominantly pediatric population.

3.14 Correlative biological studies

The process of formation of new blood vessels for tumor growth has been termed vasculogenesis and involves the recruitment of two types of bone marrow-derived progenitor cells: endothelial progenitor cells expressing VEGF receptor 2 (VEGFR2) and hematopoietic progenitor cells expressing VEGF receptor 1 (VEGFR1)[84]. The latter is considered more important in the development of metastasis[85]. Placental derived growth factor (PlGF) is a chemokine that signals through VEGFR1 and determination of PlGF levels may serve as a surrogate marker to evaluate response to therapy[86]. Circulating bone marrow-derived cells that express VEGFR1 and VEGFR2 coexpress CD133[87, 88], a possible cancer stem cell marker[89]. The integrin $\alpha_4\beta_1$ (VLA-4) is also important in the movement of these progenitor cells and is coexpressed with VEGFR1[90]. Genes belonging to *HIF* family are considered to be important regulators for the angiogenic switch that transcribes the downstream genes for angiogenesis. HIF1 subunit accumulates rapidly inside hypoxic cells due to the lack of ubiquitation and proteosomal degradation of the protein, which usually take place in normoxic cells. Both HIF-1α and -2α accumulation can drive tumor neovascularization by inducing further pro-angiogenic factors such as VEGF[91, 92].

4.0 OVERVIEW OF STUDY DESIGN/INTERVENTION

4.1 DESIGN

This is a phase II study of the combination of irinotecan, temozolomide and bevacizumab in patients with resistant NB. Patients will initially receive bevacizumab IV at 15mg/kg/dose (this is defined as Day 1 Three days later (starting day 4), they will receive concurrently, IV irinotecan at 50mg/m^2/day x 5 days plus PO temozolomide 150mg/m^2/day x 5 days. A second dose of bevacizumab will be administered 14 days after the first one(day 15). Patients will be evaluated at least once weekly for toxicity. Patients will be evaluable for toxicity if they receive at least one dose of bevacizumab even if they have not received any irinotecan or temozolomide. If patients do not experience significant toxicity (described in Section 9.8), they will commence a second cycle 4-6 weeks after the first cycle. Extent of disease evaluation will be carried out after two cycles and if there is no progressive disease and patients do not experience significant toxicity (described in Section 9.9), they may receive combination therapy up to 2 years. Responses will be evaluated after every two cycles and will be compared to historical data for the combination of irinotecan + temozolomide. Early stopping rules will be established if undue toxicities or inadequate responses are observed. Correlative studies will include assessment of circulating BM angiogenic precursors and circulating VEGF and HIF1 alpha. Schema for each cycle is shown below in Table 2. Plan for treating with more than one cycle is outlined in Figure 2.

Table 2: Schema for each cycle.

Day	Intervention
Treatment Schema for each cycle	
1	Pre treatment blood draw for angiogenic profile*; bevacizumab 15mg/kg

4	Blood draw for circulating angiogenic profile*; Irinotecan 50mg/m^2/day+ temozolomide 150mg/m^2/day
5-8	Irinotecan 50mg/m^2/day+temozolomide 150mg/m2/day. CBC and clinical chemistry including renal and liver function tests once a week.
11-15	CBC and clinical chemistry including renal and liver function tests once a week.
15	Blood draw for angiogenic profile* followed by bevacizumab 15mg/kg
18-22	CBC and clinical chemistry including renal and liver function tests once a week.
22-35	Blood draw for angiogenic profile*.
22-42	Evaluation for eligibility for second cycle (See Section 9.8)
25-29	CBC and clinical chemistry including renal and liver function tests once a week.
30-36	CBC and clinical chemistry including renal and liver function tests once a week. (if second cycle not started)
37-42	CBC and clinical chemistry including renal and liver function tests once a week. (if second cycle not started)

*Blood draw for angiogenic profile are only done during cycle 1 and cycle 2 day 1.

Patients will be removed from study if they suffer either PD or significant toxicities (as defined in Section 13). If life-threatening toxicity occurs in any patient, further accrual will be stopped pending review by the Principal Investigator. Patients may be eligible for further cycles of therapy up to a maximum of 2 years starting from first cycle. Patients who have a serious adverse event at the time of discontinuation from study treatment will continue to be followed.

4.2 Intervention

Each cycle consists of two doses of bevacizumab and five doses each of irinotecan and temozolomide (Table 2). Patients will be administered a dose of IV bevacizumab 15mg/kg on day 1 and 15 of study. Irinotecan 50mg/m^2/day IV will be administered from day 4-8 concurrently with temozolomide 150mg/m^2/day orally. The treatment schedule may require minor adjustment as clinically indicated (e.g., due to PDH closure for holidays).

Table 3: Treatment cycle

Day	Intervention
1	Bevacizumab 15mg/kg IV
4-8	Irinotecan 50mg/m^2/day+temozolomide 150mg/m^2/day
15	Bevacizumab 15mg/kg IV

Monitoring for toxicities: Clinical observation for toxicity will be undertaken at weekly outpatient visits while the patient is on study. Biochemical testing for liver and kidney function and complete blood counts will be carried out at least weekly while the patient is on study.

Safety: A number of measures will be taken to ensure the safety of patients participating in this trial. These measures will be addressed through (a) exclusion criteria (see Section 6.2), (b) early stopping rules (see Section 13.0) and (c) clinical and laboratory evaluations at weekly intervals.

Safety evaluations will consist of medical interviews, recording of adverse events, physical examinations, blood pressure, and laboratory measurements.
Specific bevacizumab-related monitoring procedures are as follows:

- 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.
- Proteinuria will be monitored by urine protein: creatinine ratio at least every 4 weeks while on study.
- 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 chemotherapy be restarted no earlier than 6 wk and bevacizumab no earlier than 8 wk after surgery).

Patients who are withdrawn for the study due to toxicity will be evaluated at least once 28-42 days after withdrawal.

Tumor response: Disease status will be evaluated after every two cycles. Patients who are withdrawn from study after one cycle will also have disease status evaluated if feasible.

Angiogenic profile: will be assessed on serial blood draws during cycle 1 on days 1, 4, 15 and once between days 22-35. Then during cycle 2 on day 1.

Plan for treatment with more than one cycle is shown in Figure 2.

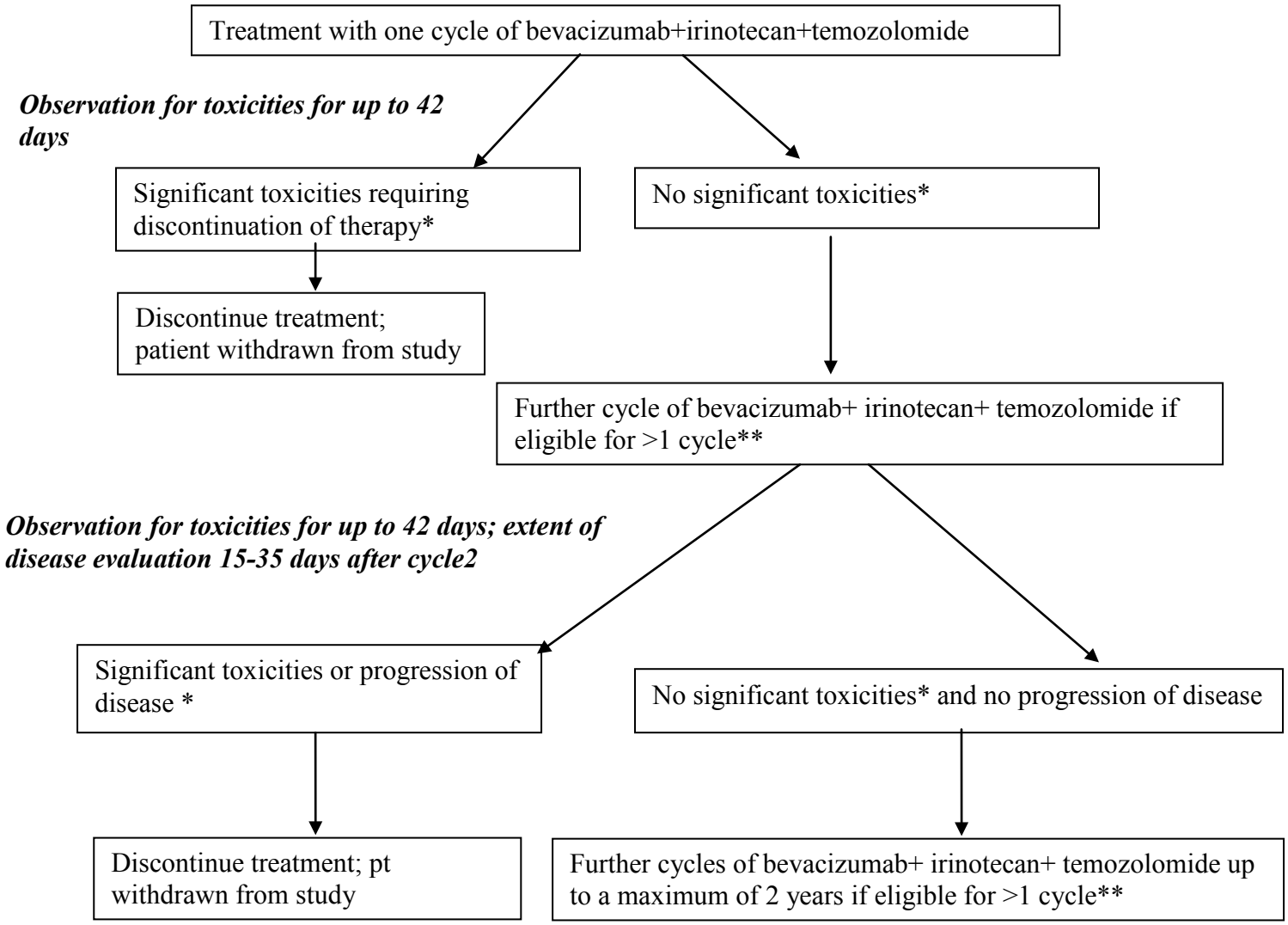

Figure 2: Algorithm for continuing treatment. * *Significant toxicities requiring discontinuation of therapy are defined in Section 13.0.* ***Eligibility for >1cycle is defined in Section 9.8*

5.0 THERAPEUTIC/DIAGNOSTIC AGENTS

5.1 **Irinotecan** (Camptosar, CPT-11)
Please refer to the package for comprehensive information.

5.1.1 *Chemistry*: Irinotecan hydrochloride trihydrate (CPT-11) is a topoisomerase I inhibitor.

5.1.2 *Formulation*: The drug is supplied in two forms: 2 mL vials containing 40 mg of drug and 5 mL vials containing 100 mg of drug. The drug is supplied in brown vials and appears as a pale-yellow-to-yellow crystalline powder and pale yellow transparent solution when reconstituted.

5.1.3 *Administration:* Irinotecan will be diluted with D5W to a total volume of up to 100 ml and infused IV over 60 minutes.

5.1.4 *Storage and Stability:* Irinotecan vials must be stored in a cool, dry place, protected from light in a locked space. It is stable to the expiration date on its labeling. After reconstitution with D5W, irinotecan is stable refrigerated for 14 days or at room temperature for 24 hours.

5.1.5 *Adverse Events:* Phase I and II studies of irinotecan have reported neutropenia and/or late diarrhea (diarrhea occurring more than 8 hours after irinotecan administration) as the dose-limiting toxicities (depending upon the schedule). Other commonly observed adverse events include nausea and vomiting, anorexia, abdominal cramping, alopecia, asthenia, lymphocytopenia, and anemia. Dehydration has occurred as a consequence of diarrhea, particularly when associated with severe vomiting. Patients may have an acute syndrome of lacrimation, diaphoresis, abdominal cramping, and diarrhea (early diarrhea) during or shortly after irinotecan administration; this syndrome is thought to be cholinergically mediated. Prophylactic or therapeutic administration of atropine should be considered in patients experiencing cholinergic symptoms. Loperamide should be readily available to begin treatment for late diarrhea (generally occurring more than 24 hours after administration of irinotecan). Sporadic cases of pulmonary toxicity, manifested as shortness of breath, non productive cough, and transient infiltrates on chest X-ray have been reported. Infrequent occurrences of mucositis or colitis (sometimes with gastrointestinal bleeding) have been observed. Occasionally, abnormalities of serum creatinine, hepatic enzymes, or thrombocytopenia have been observed. Irinotecan may cause local irritation at infusion sites.

5.1.6 *Supply:* Commercially available.

5.2 **Temozolomide** (Temodar)
Please refer to the package insert for comprehensive information.

5.2.1 *Formulation:* Temozolomide is supplied in white opaque, preservative-free, two-piece, hard gelatin capsules of the following p.o. dosage strengths: 5 mg, 20 mg, 100 mg, 140 mg, 180 mg, and 250 mg. Each capsule contains drug substance in combination with lactose, anhydrous NF, colloidal silicon dioxide NF, sodium starch glycolate NF, tartaric acid NF, and stearic acid NF. The capsule shells contain gelatin NF, titanium dioxide USP, and sodium lautyl sulfate NF.

5.2.2 *Mode of Action:* Alkylating agent of imidazotetrazinone class.

5.2.3 *Storage and Stability:* The capsules are packaged in Type I amber glass bottles (30 capsules/bottle) and should be stored at room temperature. Capsules are stable for at least 30 months when stored in amber glass bottle at this temperature.

5.2.4 *Supply:* Commercially available.

5.2.5 *Administration*: Temozolomide is administered orally.

5.2.5 *Pharmacokinetics:* Temozolomide is rapidly and completely absorbed after oral administration; peak plasma concentrations occur in 1 hour. Temozolomide is rapidly eliminated with a mean elimination half-life of 1.8 hours and exhibits linear kinetics over the therapeutic dosing range. Temozolomide has a mean apparent volume of distribution of 0.4 L/kg (%CV=13%). It is weakly bound to human plasma proteins; the mean percent bound of drug-related total radioactivity is 15%.

5.2.6 *Metabolism and Elimination:* Temozolomide is spontaneously hydrolyzed at physiologic pH to the active species, 3-methyl-(triazen-1-yl) imidazole-4-carboxamide (MTIC) and to temozolomide acid metabolite. MTIC is further hydrolyzed to 5-amino-imidazole-4-carboxamide (AIC), which is known to be an intermediate in purine and nucleic acid biosynthesis and to methylhydrazine, which is believed to be the active alkylating species. Cytochrome P450 enzymes play only a minor role in the metabolism of temozolomide and MTIC. Relative to the AUC of temozolomide, the exposure to MTIC and ACI is 2.4% and 23% respectively. About 38% of the administered temozolomide total radioactive dose is recovered over 7 days; 37.7% in urine and 0.8% in feces. The majority of the recovery of radioactivity in urine is as unchanged temozolomide (5.6%), AIC (12%), temozolomide acid metabolite (2.3%), and unidentified polar metabolites(s) (17%). Overall clearance of temozolomide is about 5.5 L/hr/$m^{2.}$

5.2.7 *Known Potential Adverse Events:* (a) Hematologic: Thrombocytopenia, leukopenia, myelodysplastic syndrome (b) Gastrointestinal: Nausea, vomiting, anorexia (c) Hepatic: Elevated liver enzymes (reversible) (d) Skin: Rash. (e) Other: Constipation, diarrhea, stomatitis, fatigue, decreased performance status, headache

5.2.8 *Pregnancy:* Temozolomide is potentially mutagenic and should be handled with appropriate precautions by both staff and patients. Procedures for proper handling and

disposal of anticancer drugs should be considered. Temozolomide may cause fetal harm when administered to a pregnant woman. There are no adequate and well-controlled studies in pregnant women. If this drug is used during pregnancy, or if the patient becomes pregnant while taking this drug, the patient should be apprised of the potential hazard to the fetus. Women of childbearing potential should be advised to avoid becoming pregnant during therapy with temozolomide. Men receiving temozolomide should be directed to use effective contraception while they are being treated.

5.3 Bevacizumab (Avastin)

Please refer to the bevacizumab Investigator Brochure for further details and molecular characterization.

5.3.1 Formulation: **:**Bevacizumab is a clear to slightly opalescent, colorless to pale brown, sterile, pH 6.2 solution for IV infusion. It is supplied in 100 mg and 400 mg preservative-free, single-use vials to deliver 4 mL or 16 mL of bevacizumab (25 mg/mL). It contains α-trehalose dihydrate sodium phosphate (monobasic, monohydrate), sodium phosphate (dibasic, anhydrous), polysorbate 20, and Water for Injection, USP. Vials are for single use only. Bevacizumab being administered in this study is intended for use only in clinical trials. It is expected to be very similar in safety and activity to the commercially marketed drug (Avastin), but it is possible that some differences may exist.

5.3.2 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.

5.3.3 Preparation of bevacizumab infusion: The necessary amount of bevacizumab should be withdrawn and diluted in 0.9% Sodium Chloride Injection, USP to make a final volume of 100 mL. Unused portion left in the vial should be discarded, as the product contains no preservatives. Vials are for single use only. Bevacizumab infusions should not be administered or mixed with dextrose solutions. Diluted bevacizumab solutions for infusion may be stored at 2-8°C (36-46°F) for up to 8 hours.

5.3.4 Administration: Administration will be as a continuous IV infusion. Anaphylaxis precautions should be observed during study drug administration. 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. It should not be administered as IV push or bolus. Bevacizumab infusion should be interrupted in all patients with severe reactions and appropriate medical therapy administered. The line should be flushed after infusion is complete.

5.3.5 Toxicity: Bevacizumab toxicities are described in detail in section 3.8 and are also summarized below in Table 4.

Table 4: Bevacizumab toxicities

Likely (>20% Frequency)	Less Likely (≤ 20%)	Rare (<3% Frequency)
reproductive system and breast disorders - other (ovarian failure)[j]; hypertension	anemia, febrile neutropenia; supraventricular tachycardia; vertigo; abdominal pain, colitis, constipation, diarrhea, dyspepsia, gastrointestinal hemorrhage[b], gastrointestinal obstruction[c], ileus; mucositis oral, nausea, vomiting; fatigue, infusion related reaction, non-cardiac chest pain, pain; allergic reaction; infection[f], infection 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)[g], myalgia, osteonecrosis of jaw[h]; dizziness, headache, peripheral sensory neuropathy[i], syncope; hematuria, proteinuria; vaginal hemorrhage; allergic rhinitis, cough, dyspnea, epistaxis, hoarseness; pruritus, rash maculo-papular, urticaria; thromboembolic event	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[a], gastrointestinal perforation[d], gastrointestinal ulcer[e]; anaphylaxis; gastrointestinal anastomotic leak; intracranial hemorrhage, ischemia cerebrovascular, reversible posterior leukoencephalopathy syndrome; acute kidney injury, 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)[k]

[a] 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.

[b] 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.

[c]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.

[d]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.

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

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

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

[h]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.

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

[j]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.

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

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

BLOOD AND LYMPHATIC SYSTEM DISORDERS - Blood and lymphatic system disorders - Other (idiopathic thrombocytopenia purpura); Disseminated intravascular coagulation
CARDIAC DISORDERS - Pericardial effusion
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS - Gait disturbance; Sudden death NOS
HEPATOBILIARY DISORDERS - Hepatic failure
INFECTIONS AND INFESTATIONS - Infections and infestations - Other (aseptic meningitis)
INVESTIGATIONS - Platelet count decreased
METABOLISM AND NUTRITION DISORDERS - Hyponatremia
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS - Musculoskeletal and connective tissue disorder - Other (aseptic necrotic bone); Musculoskeletal and connective tissue disorder - Other (myasthenia gravis)
NERVOUS SYSTEM DISORDERS - Dysgeusia; Peripheral motor neuropathy; Seizure
PSYCHIATRIC DISORDERS - Confusion
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS - Adult respiratory distress syndrome; Pneumonitis; Pneumothorax; Pulmonary hypertension

SKIN AND SUBCUTANEOUS TISSUE DISORDERS - Palmar-plantar erythrodysesthesia syndrome; Skin ulceration

5.3.6 Source: Bevacizumab will be supplied by Genentech. Patients will not be charged for this agent.

6.0 CRITERIA FOR SUBJECT ELIGIBILITY

Patients with relapsed or refractory neuroblastoma will be eligible for the study.

6.1 Subject Inclusion Criteria

- Patients must have the diagnosis of NB in accordance with the International Criteria, i.e., either histopathology (confirmed by the MSKCC Department of Pathology) or BM involvement plus elevated urinary catecholamines.
- Must have a history of tumor progression or recurrence or failure to achieve complete response with standard therapy.
- Patients must have evaluable (microscopic marrow metastasis, MIBG or PET scans) or measurable (CT, MRI) disease.
- Patients of all ages are eligible.
- Prior Therapy: At least 2 weeks should have elapsed since any biologic therapy. Three weeks should have elapsed since last dose of chemotherapy.
- Minimum life expectancy of eight weeks.
- Signed informed consent indicating awareness of the investigational nature of this program.

6.2 Subject Exclusion Criteria

- Severe major organ toxicity. Renal, cardiac, hepatic, pulmonary, gastrointestinal and neurologic toxicity should all be grade 2 or less (per NCI CTC version 4.0 criteria). Specifically, serum creatinine should be ≤3 x upper limit of normal (ULN), serum AST and ALT ≤5 x ULN, serum bilirubin ≤ 3 x ULN, LV shortening fraction should be ≥15%.
- Patients with myelosuppression are not excluded if ANC ≥ 500/uL. Platelet count should be > 35,000/ul and hemoglobin should be > 8gm/dl. Patients should not have received filgrastim, platelet or red blood cell transfusions for 2 days prior to achieving the above ANC, platelet and hemoglobin levels.
- Patients with documented chronic non-healing wound, ulcer or bone fracture
- Surgical procedures.
    - Patients who have undergone major surgery <28 days prior to beginning therapy with bevacizumab are excluded.
    - Patients must be least 24 hours from having after surgical procedures such as placement of central catheter.

- Patients <7days from minor surgeries (e.g. fine needle or core biopsies) and/or the unhealed wounds from these procedures are excluded.
- Patients will be excluded if major surgery (e.g. abdominal or thoracic surgery for resection of tumor) is anticipated during the course of the study.

- Known bleeding diathesis or coagulopathy (in the absence of therapeutic anticoagulation)
- Thrombosis: patients must not have had a deep venous or arterial thrombosis (non-central venous catheter related) within the last three months prior to study entry. Patients with cerebrovascular accident or transient ischemic attack within 6 months of therapy are excluded. Patients with history of peripheral vascular disease, myocardial infarction or unstable angina are excluded.
- History of abdominal fistula, gastrointestinal perforation, or intra-abdominal abscess within 6 months prior to study entry.
- Known CNS metastases, except for treated brain metastasis. Treated brain metastases are defined as having no evidence of progression or hemorrhage after treatment and no ongoing requirement except 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 radiotherapy, chemotherapy or immunotherapy Patients with CNS metastases treated by neurosurgical resection or biopsy performed within 3 months of treatment will be excluded
- Proteinuria: Urine protein: creatinine ratio ≥ 1.0
- Uncontrolled (lasting >24 hrs on antihypertensive medication) hypertension as defined by age-appropriate criteria. Hypertension is defined as average systolic blood pressure and/or diastolic blood pressure that is ≥95th percentile for gender, age, and height on ≥3 occasions[93]. 95th percentiles for gender, age and height are provided in Appendix A. For patients ≥18 years of age, hypertension is defined as systolic blood pressure >150mmHg and/or diastolic blood pressure >100mmHg.
- Prior history of hypertensive crisis or hypertensive encephalopathy
- History of hypersensitivity to any component of bevacizumab
- History of hemoptysis (≥ 1/2 teaspoon of bright red blood per episode) within 1 month prior to Day 1
- Active serious infections not controlled by antibiotics.
- Pregnant women are excluded for fear of danger to the fetus. Therefore negative pregnancy test is required for all women of child-bearing age, and appropriate contraception is used during the study period.
- Inability or unwillingness to comply with protocol requirements.

7.0 RECRUITMENT PLAN

Patients will be offered the opportunity to participate in this trial if they have NB and fulfilled the eligibility criteria. The opportunity to participate will be offered to all patients, including females and minority groups. Pregnant women will be excluded for fear of danger to the fetus. Informed consent will be obtained from the patients or their legal guardians by an investigator authorized to obtain consent. Patients will not receive any payment for their participation in this study.

8.0 PRETREATMENT EVALUATION

- Complete history including history of prior thromboembolic episodes, stroke, non-healing ulcers or fractures, myocardial infarction, surgical procedures
- Physical examination including blood pressure measurement
- CBC with differential and platelet count
- Urine testing for proteinuria
- CT or MRI of head with contrast
- Clinical chemistry (including serum sodium, potassium, chloride, bicarbonate, calcium,)
- Liver function tests including ALT, AST and serum bilirubin
- Renal function tests including BUN and serum creatinine
- Echocardiogram
- Serum Beta-HCG in females of child-bearing age
- Investigations for evaluating disease possibly including: BM aspirations and biopsy, CT or MRI of suspected sites of disease, bone scan, MIBG scan, PET scan and/or urine catecholamines
- BM testing for quantifying residual disease by reverse transcriptase polymerase chain reaction (RT-PCR) (research studies; only if BM studies were done as part of pre-treatment disease.)

Pretreatment evaluation should be carried out within 6 weeks (preferably within 4 weeks) of patient being enrolled on study.

9.0 TREATMENT/INTERVENTION PLAN

9.1 General Outline

This is a phase II study of the combination of irinotecan, temozolomide and bevacizumab in patients with resistant NB. Patients will initially receive bevacizumab at 15mg/kg/dose IV (defined as Day1). Three days later (starting day 4), they will receive concurrently, IV irinotecan at 50mg/m^2/day x 5 days plus PO temozolomide 150mg/m^2/day x 5 days. A second dose of bevacizumab will be administered 14 days after the first one (day 15). Patients will be evaluated at least once weekly for toxicity. Patients will be evaluable for toxicity if they receive at least one dose of bevacizumab even if they have not received any irinotecan or temozolomide. If patients do not experience significant toxicity (described in Section 9.8), they will commence a second cycle 4-6 weeks after the first cycle. Extent of disease evaluation will be carried out after two cycles and if there is no progressive disease and patients do not experience significant toxicity (described in Section 9.9), they may continue to receive therapy up to a maximum of two years. Responses will be evaluated after every two cycles and will be compared to historical data for the combination of irinotecan + temozolomide. Enrollment on the study will be halted if undue toxicities or inadequate responses are observed (described in Section 14.2). Correlative studies will include assessment of circulating BM angiogenic precursors and circulating VEGF and

HIF1 alpha. Schema for each cycle is shown below in Table 5. Plan for treating with more than one cycle is outlined in Figure 2.

Table 5: Schema for each cycle

Day	Intervention
Treatment Schema for each cycle	
1	Pre treatment blood draw for angiogenic profile*; bevacizumab 15mg/kg
4	Blood draw for circulating angiogenic profile*
4-8	Irinotecan 50mg/m^2/day+temozolomide 150mg/m2/day. CBC and clinical chemistry including renal and liver function tests once a week.
11-15	CBC and clinical chemistry including renal and liver function tests once a week.
15	Blood draw for angiogenic profile* followed by bevacizumab 15mg/kg
18-22	CBC and clinical chemistry including renal and liver function tests once a week.
22-35	Blood draw for angiogenic profile*.
22-42	Evaluation for eligibility for second cycle (See Section 9.8)
25-29	CBC and clinical chemistry including renal and liver function tests once a week.
30-36	CBC and clinical chemistry including renal and liver function tests once a week. (if second cycle not started)
37-42	CBC and clinical chemistry including renal and liver function tests once a week. (if second cycle not started)

*Blood draws for angiogenic profile for research purposes and are done only during cycle 1 and cycle 2 day 1.

Patients will be removed from study if they suffer either progressive disease or significant toxicities (as defined in Section 13). If life-threatening toxicity occurs in any patient, further accrual will be stopped pending review by the Principal Investigator. Patients may be eligible for further cycles of therapy up to a maximum of two years. Patients who have a 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.

9.2 Treatment schedule

The following treatment schedule will be followed for each cycle of therapy.

Table 6: Treatment cycle

Day	Intervention
1	Bevacizumab 15mg/kg IV
4-8	IV Irinotecan 50mg/m^2/day+oral temozolomide 150mg/m^2/day

15	Bevacizumab 15mg/kg IV

The treatment schedule may require minor adjustment by ±1 day as clinically indicated (e.g. due to PDH closure for holidays or due to inclement weather).

9.3 Administration of bevacizumab

The dose of bevacizumab is fixed at 15mg/kg/dose. For each cycle, two doses will be administered IV on each of days 1 and 15. Each dose will be administered over 30 to 90 minutes as described in Section 5.2.

9.4 Administration of irinotecan

Irinotecan will be administered as an IV infusion over 60±15 minutes. Anti-emetics will be administered as necessary.

Irinotecan may be administered at a location more convenient for the patient. In such cases, adequate documentation must be sent to MSKCC in a timely fashion and in compliance with all study guidelines and requirements.

Documentation will include:

- Patient Name
- Patient Date of Birth
- Dates of Treatment
- Dosage of Treatment
- Facility Name
- Clinician Signature and Name

9.5 Administration of temozolomide

Temozolomide will be administered orally. The dose of temozolomide will be adjusted such that it can be administered using available capsules i.e. will be rounded off to the nearest 5 mg. If the patient vomits within approximately 30 minutes after receiving temozolomide, the treatment dose may be re-administered.

9.6 Angiogenic profile

Blood draws for angiogenic profile are for research purposes and are optional. The following parameters of angiogenesis will be monitored in patients' blood samples:

- Circulating BM derived progenitor cells expressing CD133, VEGFR1 and VEGFR2, and integrin $\alpha_4\beta_1$ by immunocytology
- Plasma VEGF and PlGF and HIF1α using ELISA assays
- RNA and DNA will be extracted and stored for subsequent testing for other angiogenic markers

Blood will be sampled during cycle 1 on days 1, 4, 15, and once between days 22 and 35 where feasible. Blood will also be sampled during cycle 2 day 1 where feasible. Samples on days 1 and 15 will be obtained before bevacizumab administration. Tests for angiogenic profile will be

carried out in Dr Nai-Kong Cheung's laboratory (Z-727G). Blood draws for angiogenic profile are optional and specific consent will be obtained from the patient.

9.7 Toxicities requiring discontinuation of therapy

The following toxicities will necessitate discontinuation of therapy for a particular patient

- Grade 4 toxicity, other than myelosuppression (and its sequelae namely thrombocytopenia-related bleeding and infection), clearly attributable to irinotecan, temozolomide or bevacizumab.
- wound dehiscence requiring medical or surgical intervention,
- arterial thromboembolic event,
- GI perforation,
- CNS hemorrhage,
- reversible posterior encephalopathy (confirmed by MRI)
- tracheo-esophageal fistula.
- ≥Grade 3 allergic reaction,
- ≥Grade 3 hypertension,
- ≥Grade 3 venous thromboembolism
- grade 1 pulmonary hemorrhage
- nephrotic syndrome
- grade ≥ 3 bowel obstruction that has not fully recovered despite medical or surgical intervention
- >grade 2 congestive cardiac failure

9.8 Eligibility for second cycle of therapy

Patients will be eligible for a second cycle of chemotherapy if they fulfill the following criteria: (a) They do not experience any of the toxicities defined in Section 9.7 until day 42 of therapy or until recovery from myelosuppression (myelosuppression is defined as ANC<500/uL and platelet count <20,000/uL) whichever is earlier. Based on our prior experience with the combination of irinotecan+temozolomide, subsequent cycles could be commenced 3-5 weeks apart even in patients whose pre-treatment platelet counts were <30,000/uL. Therefore, we do not expect prolonged myelosuppression. However, if patients have not recovered from myelosuppression by day 42, they will not be eligible for further therapy.
(b) They continue to not have any major organ toxicity as evaluated by clinical examination and blood draws between days 22-42. Specifically: serum creatinine should be ≤3 x upper limit of normal (ULN), serum AST and ALT ≤5 x ULN, serum bilirubin ≤ 3 x ULN, ANC ≥ 500/uL, platelet count > 35,000/ul, hemoglobin > 8gm/dl and urine protein: creatinine ratio < 1.0. (c) They continue to meet eligibility criteria defined in Section 6, with the exception that patients who are found to be in remission after an extent of disease evaluation may continue on treatment for up to 2 years. Second and subsequent cycles can only be commenced at a minimum of 14 and a maximum of 28 days after the previous dose of bevacizumab i.e. the second cycle cannot commence before day 29 or after day 43.

9.9 Eligibility for continuing therapy beyond second cycle

Patients who do not have disease progression as evaluated on day 15-35 after the second cycle and continue to meet eligibility criteria defined in section 9.8 can continue to receive bevacizumab plus irinotecan and temozolomide for a maximum of two years. The algorithm shown in figure 3 will continue to be followed. Extent of disease evaluations will be performed after every two cycles. Subsequent cycles can only be commenced at a minimum of 14 and a maximum of 28 days after the previous dose of bevacizumab i.e. the second cycle cannot commence before day 29 or after day 43.

9.10 Use of G-CSF

G-CSF (5-10ug/kg) may be used as clinically indicated.

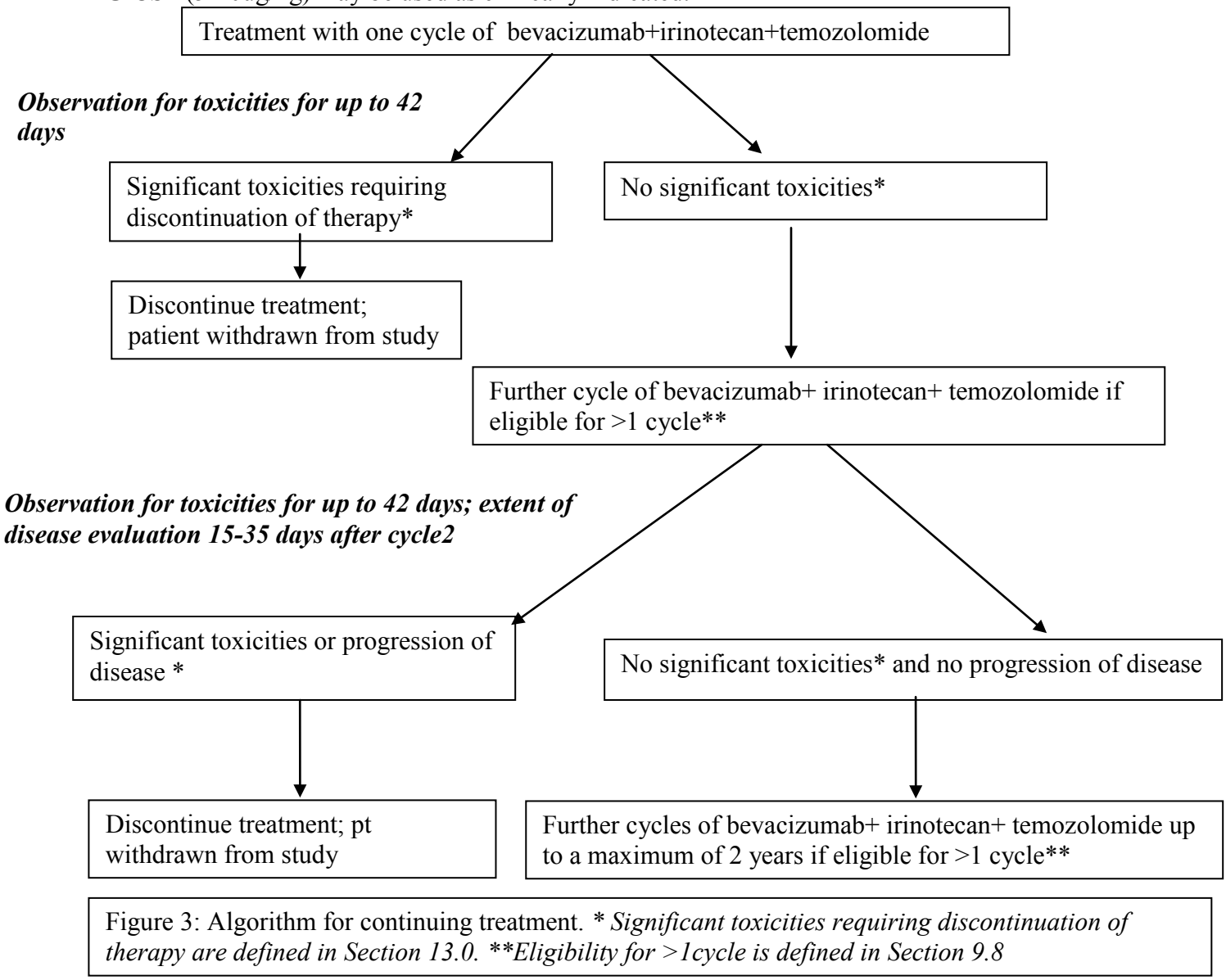

Figure 3: Algorithm for continuing treatment. * *Significant toxicities requiring discontinuation of therapy are defined in Section 13.0.* ***Eligibility for >1cycle is defined in Section 9.8*

Patients may receive radiation therapy to sites of disease that are not being assessed for disease or response evaluation, at the discretion of the PI.

10.0 EVALUATION DURING TREATMENT/INTERVENTION

The following evaluations will be carried out during treatment:

Table 7: Evaluations during treatment

Evaluation	Timing
A. Pretreatment evaluation	
Clinical history and exam including blood pressure	Within 6 weeks of enrollment
CBC	
Urine testing for proteinuria	
Clinical chemistry (including serum sodium, potassium, chloride, bicarbonate, calcium)	
Liver function tests including ALT, AST and serum bilirubin	
Renal function tests including BUN and serum creatinine	
Tests for disease evaluation possibly including BM, MIBG, Bone scan, PET scan CT/MRI and/or LDH	
CT or MRI of head	
Echocardiogram	
Serum Beta-HCG in females of child-bearing age;	
BM testing for quantifying residual disease by RT-PCR (research studies)	
B. Evaluations while on treatment	
Clinical exam including blood pressure	Weekly
CBC	Weekly
Serum electrolytes, BUN, creatinine, ALT, total bilirubin, AST	Weekly
Urine protein to creatinine ratio	Every four weeks while on study
X-ray knee	At end of therapy
Tests for disease evaluation possibly including BM, MIBG, Bone scan, PET scan CT/MRI and/or LDH	After two cycles of therapy and then after every two cycles until a maximum of two years
BM studies for minimal residual disease evaluation by RT-PCR	At times of BM testing as above
C. Evaluations after protocol treatment is completed	
Xray knee	Once three to six months after therapy
Tests for disease evaluation	As indicated

11.0 TOXICITIES/SIDE EFFECTS

This combination has not been previously administered to children and there are limited data on the use of bevacizumab in children. Toxicities are graded using the NCI Scale CTCAE (Version 4.0).

11.1 Toxicities associated with irinotecan

Common/expected: Diarrhea that may lead to dehydration, abdominal cramping, nausea and vomiting, anorexia, sweating and flushing, alopecia, asthenia, myelosuppression, lacrimation nd diaphoresis as part of a cholinergically mediated syndrome

Rare: interstitial lung disease, nephrotoxicity, hepatotoxicity, mucositis, colitis,gastrointestinal bleeding, local irritation at infusion sites.

11.2 Toxicities associated with temozolomide

Common/expected: Nausea, vomiting, anorexia, headache, constipation, fatigue, myelosuppression
Rare: seizures, opportunistic infection, secondary malignancy, hepatotoxicity, hepatic failure, rash, constipation, diarrhea, and mucositis.

11.3 Toxicities associated with bevacizumab

Bevacizumab toxicities are described in detail in Section 3.8 and summarized in Table 8 below. Acute toxicities reported with bevacizumab are those expected with administration of a humanized monoclonal antibody and include headache, fever, fatigue, nausea, vomiting, arthralgia and rash.

Table 8: Bevacizumab toxicities

Likely (>20% Frequency)	Less Likely (≤ 20%)	Rare (<3% Frequency)

reproductive system and breast disorders - other (ovarian failure)[j]; hypertension	anemia, febrile neutropenia; supraventricular tachycardia; vertigo; abdominal pain, colitis, constipation, diarrhea, dyspepsia, gastrointestinal hemorrhage[b], gastrointestinal obstruction[c], ileus; mucositis oral, nausea, vomiting; fatigue, infusion related reaction, non-cardiac chest pain, pain; allergic reaction; infection[f], infection 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)[g], myalgia, osteonecrosis of jaw[h]; dizziness, headache, peripheral sensory neuropathy[i], syncope; hematuria, proteinuria; vaginal hemorrhage; allergic rhinitis, cough, dyspnea, epistaxis, hoarseness; pruritus, rash maculo-papular, urticaria; thromboembolic event	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[a], gastrointestinal perforation[d], gastrointestinal ulcer[e]; anaphylaxis; gastrointestinal anastomotic leak; intracranial hemorrhage, ischemia cerebrovascular, reversible posterior leukoencephalopathy syndrome; acute kidney injury, 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)[k]

[a] 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.

[b] 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.

[c] 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.

[d]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.

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

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

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

[h]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.

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

[j]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.

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

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

BLOOD AND LYMPHATIC SYSTEM DISORDERS - Blood and lymphatic system disorders - Other (idiopathic thrombocytopenia purpura); Disseminated intravascular coagulation
CARDIAC DISORDERS - Pericardial effusion
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS - Gait disturbance; Sudden death NOS
HEPATOBILIARY DISORDERS - Hepatic failure
INFECTIONS AND INFESTATIONS - Infections and infestations - Other (aseptic meningitis)
INVESTIGATIONS - Platelet count decreased
METABOLISM AND NUTRITION DISORDERS - Hyponatremia
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS - Musculoskeletal and connective tissue disorder - Other (aseptic necrotic bone); Musculoskeletal and connective tissue disorder - Other (myasthenia gravis)
NERVOUS SYSTEM DISORDERS - Dysgeusia; Peripheral motor neuropathy; Seizure
PSYCHIATRIC DISORDERS - Confusion
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS - Adult respiratory distress syndrome; Pneumonitis; Pneumothorax; Pulmonary hypertension
SKIN AND SUBCUTANEOUS TISSUE DISORDERS - 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.

11.4 Management of toxicity associated with bevacizumab.
There are no reductions in the bevacizumab dose. Dose management of bevacizumab due to adverse events is described in Table 9, section 11.5.
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.
Infusion Reaction: Emergency support for possible anaphylaxis will be available. 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 will be discontinued from 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 in Table 10. Regardless of the reason for holding study drug treatment, the maximum allowable length of treatment interruption is 2 months.

11.5 All serious adverse events will be reported to the Institutional Review Board of MSKCC, FDA, Genentech Drug Safety.

Table 9: Bevacizumab Dose Management Due to Adverse Events

Event	**Action to be Taken**
Allergic reaction	
Grade1/2	No modifications
Grade 3/4	Discontinue bevacizumab; patient withdraws from study
Hypertension	
Grade 1/2 events	No modifications
Grade 3	If not controlled to age-appropriate levels with medication, discontinue bevacizumab
Grade 4	Discontinue bevacizumab; patient withdraws from study
Hemorrhage	
CNS hemorrhage (any grade)	Discontinue bevacizumab; patient withdraws from study
Grade ≥ 2 pulmonary hemorrhage	Discontinue bevacizumab; patient withdraws from study
Grade 1 pulmonary or ≥ grade 2 non pulmonary hemorrhage	No dose modifications
≥ Grade 3 non-pulmonary, non-CNS hemorrhage	Discontinue bevacizumab; patient withdraws from study
Venous Thrombosis	

Grade 1/2	No dose modifications
Grade 3/4	Discontinue bevacizumab; patient withdraws from study
Arterial Thromboembolic event (Angina, myocardial infarction, transient ischemic attack, cerebrovascular accident, and any other arterial thromboembolic event) Any grade	Discontinue bevacizumab; patient withdraws from study
Congestive Heart Failure (Left ventricular systolic dysfunction)	
1/2 events	No dose modifications
Grade 3/4	Discontinue bevacizumab; patient withdraws from study
Proteinuria	
Grade 1/2	No dose modifications
Grade 3 (UPC> 3.5, urine collection > 3.5 g/24 hr, or dipstick 4+)	Hold bevacizumab treatment until ≤ Grade 2, as determined by either UPC ratio ≤ 3.5 or 24 hr collection ≤ 3.5 g
Grade 4 (nephrotic syndrome)	Discontinue bevacizumab; patient withdraws from study
Bowel Obstruction	
Grade 1	Continue patient on study for partial obstruction NOT requiring medical intervention.
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.
Fistula	
TE fistula any grade	Discontinue bevacizumab; patient withdraws from study
Grade 4 fistula	Discontinue bevacizumab; patient withdraws from study
Wound dehiscence requiring medical or surgical therapy	Discontinue bevacizumab; patient withdraws from study
Reversible Posterior Leukoencephalopathy confirmed by MRI any grade	Discontinue bevacizumab; patient withdraws from study
Other Unspecified Bevacizumab-Related Adverse Events	
Grade 3	Hold bevacizumab until recovery to ≤ Grade 1
Grade 4	Discontinue bevacizumab.

12.0 CRITERIA FOR THERAPEUTIC RESPONSE/OUTCOME ASSESSMENT

12.1 Response duration is calculated from the first day of treatment with bevacizumab.

12.2 Patients developing progressive disease will be considered a treatment failure under this protocol.

12.3 Therapeutic response in patients with NB will be assessed using the International Neuroblastoma Response Criteria[94] which are shown below.

International Neuroblastoma Response Criteria:

- Complete remission/response: Complete disappearance of all evidence of disease.
- Very good partial remission/response: >90% decrease in volume of primary tumor and in all other markers of disease, except bone scan (if initially abnormal) stable or improved; BM must be free of disease
- Partial remission/response: 50 to 90% decrease in measurable, and no new, lesions, except bone scan stable or improved (if initially abnormal). Bones and BM: no more than 1 positive BM site allowed.
- Mixed response (MR): No new lesions; >50% reduction of any measurable lesion (primary or metastases) with <50% reduction in any other; <25% increase in any existing lesion.
- No response (NR; stable disease): <50% reduction in all tumor markers; no new lesions; <25% increase in any existing lesion.
- Progressive disease (PD): Any new lesion; increase of any measurable lesion by >25%; previous negative marrow positive for tumor.

13.0 CRITERIA FOR REMOVAL FROM STUDY

Patients will come off study at any time if disease progression is observed. If patients are removed from study, further patients will not be added as replacements.

13.1Patients will be withdrawn from study if the following toxicities occur:

13.1.1 If at any time the patient develops life-threatening grade 4 toxicity, other than myelosuppression, clearly attributable to irinotecan, temozolomide or bevacizumab, he/she will be removed from study.

- If the patient develops any grade of the following toxicities, the patients will be removed from study:
- wound dehiscence requiring medical or surgical intervention,
- arterial thromboembolic event,
- GI perforation,
- CNS hemorrhage,
- reversible posterior encephalopathy (confirmed by MRI)
- tracheo-esophageal fistula.

- ≥Grade 3 allergic reaction,
- ≥Grade 3 hypertension,
- ≥Grade 3 venous thromboembolism
- > grade 1 pulmonary hemorrhage
- nephrotic syndrome
- >grade 2 congestive cardiac failure.
- grade ≥ 3 bowel obstruction that has not fully recovered despite medical or surgical intervention
- ANC<500/uL and/or platelet count <20,000/uL on day 43 of treatment

13.2 Patients who are removed from the study due to adverse events will be treated and followed according to established, accepted medical practice. 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. All pertinent information concerning the outcome of such treatment will be entered in the Case Report Form or on the Serious Event Report, as applicable.

13.3 If at any time the patient is found to be ineligible for the protocol as designated in the section on Criteria for Patient/Subject Eligibility (i.e., a change in diagnosis), the patient will be removed from the study

13.4 Withdrawal of consent. The patient's desire to withdraw from the study may occur at any time. The investigator should carefully consider whether the patient's withdrawal of consent is due to an adverse event, and if so, record the adverse event as the reason for withdrawal.

13.5 If at any time the patient is non-compliant with therapy or requests withdrawal from the study, he/she will be removed from study.

13.6 If at any time, the principal investigator determines that it is no longer safe for the subject to continue therapy, the subject will be removed from study

14.0 BIOSTATISTICS

This is a phase II study of the combination of irinotecan, temozolomide and bevacizumab in patients with resistant NB. The primary endpoint is response rate (CR+PR) by INSS response criteria. The secondary endpoints are toxicity, time to progression, and post-treatment angiogenic markers.

14.1 Study Design and Sample Size

The Simon's optimal two-stage design will be used for this phase II study. The primary endpoint is response rate (CR+PR) by INSS response criteria. Response assessment will be based on the best response over the course of four cycles. The undesirable response rate is set at 20% based on

prior studies. The desirable response rate is set at 35%. The type I error is set at 10%, and type II error at 10%. In the first stage 27 patients will be enrolled: if response (CR+PR) is observed in ≤5 patients, the combination of irinotecan, temozolomide and bevacizumab will be considered ineffective and the study will be stopped. If response (CR+PR) is observed in ≥6 patients, the study will continue to the next stage and an additional 34 patients will be enrolled. The combination will be considered effective if ≥17/63 patients show response. If disease progresses in patients prior to their completing four cycles, treatment will be considered to have failed. Such patients will not be replaced on study.

14.2 Stopping Rules for Toxicity

Since the combination of irinotecan, temozolomide and bevacizumab has not been previously used in children, we have inserted early stopping rules to avoid undue toxicity. Toxicity will be graded using CTC version 4.0.

In our previous reports, grade 4 myelosuppression and grade 3 diarrhea were expected complications of treatment with irinotecan and temozolomide. 1/49 patients also developed grade 3 hepatic transaminitis. In our previous experience with the combination of irinotecan and temozolomide, we were able to administer successive cycles 3-5 weeks apart even in heavily pretreated patients with pre treatment platelet counts <35,000/uL.

In order to reduce patient risk, the study design includes early stopping during the first cycle of treatment in the event of excessive (1) grade 4 diarrhea, or (2) severe myelosuppression defined as failure to reach ANC ≥500/uL and/or platelet count >20,000 by day 43 (day 1 is defined as each cycle), (3) grade 3 or 4 toxicity (other than myelosuppression, infection secondary to myelosuppression or diarrhea) clearly related to the combination of irinotecan, temozolomide and bevacizumab. The stopping rules for excessive toxicity and the corresponding power calculations are given in the table below.

Table 10: Stopping Rules

Toxicity Type	Number of toxicities needed to stop the study	Probability of toxicity type	Probability of early study stopping
Grade 4 diarrhea	4 within the first 12 patients 6 within the first 27 patients 8 within the first 44 patients 11 within 63 patients	0.1	0.11
		0.2	0.80
Severe myelosuppression	4 within the first 12 patients 6 within the first 27 patients 8 within the first 44 patients 11 within 63 patients	0.1	0.11
		0.2	0.80

Other grade 3 or 4 toxicity	4 within the first 12 patients 6 within the first 27 patients 8 within the first 44 patients 11 within 63 patients	0.1	0.11
		0.2	0.80

14.3 Patient Accrual

Review of accrual patterns from previous neuroblastoma studies indicates that 1-2 patients per month may be accrued. This will permit completion of the first phase of the study in approximately two years. If the study proceeds to the second phase, it is anticipated that an additional four years will be required to complete the entire study.

14.4 Analysis Plan

Overall response rate (CR+PR) will be calculated and its 90% confidence interval provided. Toxicity events will be listed. Time to progression will be estimated using the Kaplan-Meier curve. The 95% confidence interval will be provided for 3-year time to progression. Angiogenic profile will be assessed by measuring plasma VEGF, HIF1 alpha and PIGF levels, and circulating CD133 positive cells in the blood, before and after treatment with bevacizumab. The average before vs. after treatment change will be estimated for each angiogenic marker and will be evaluated using the Wilcoxon signed rank test. The analysis of correlative markers will only be embarked upon if the study proceeds to the second stage.

Patients will be included for toxicity assessment as long as they have received one dose of bevacizumab. Patients will be evaluable for response if they have received at least two doses of bevacizumab and 5 doses each of irinotecan and temozolomide. If patients receive only one cycle of therapy, response evaluation will be carried out prior to their coming off study. Patients who do not complete at least one cycle of therapy and therefore not evaluable for response will be replaced on study.

15.0 SUBJECT REGISTRATION AND RANDOMIZATION PROCEDURES

15.1 Research Participant Registration

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

Obtain informed consent, by following procedures defined in section entitled Informed Consent Procedures.

During the registration process registering individuals will be required to complete a protocol specific Eligibility Checklist.

All participants must be registered through the Protocol Participant Registration (PPR) Office at Memorial Sloan-Kettering Cancer Center. PPR is available Monday through Friday from 8:30am – 5:30pm at 646-735-8000. Registrations must be submitted via the PPR Electronic Registration System (http://ppr/). The completed signature page of the written consent/RA, a completed Eligibility Checklist and other relevant documents must be uploaded via the PPR Electronic Registration System.

16.0 DATA MANAGEMENT ISSUES

A Research Study Assistant (RSA) will be assigned to the study. The responsibilities of the RSA include project compliance, data collection, abstraction and entry, data reporting, regulatory monitoring, problem resolution and prioritization, and coordinate the activities of the protocol study team. The data collected for this study will be entered into a secure database. Source documentation will be available to support the computerized patient record.

16.1 Quality Assurance

Weekly registration reports will be generated to monitor patient accruals and completeness of registration data. Routine data quality reports will be generated to assess missing data and inconsistencies. Accrual rates and extext and accuracy of evaluations and follow-up will be monitored periodically throughout the study period and potential problems will be brought to the attention of the study team for discussion and action

Random-sample data quality and protocol compliance audits will be conducted by the study team, at a minimum of two times per year, more frequently if indicated.

16.2 Data and Safety Monitoring

The Data and Safety Monitoring (DSM) Plans at Memorial Sloan-Kettering Cancer Center were approved by the National Cancer Institute in September 2001. 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/researchers/dsm/index.html. The DSM Plans at MSKCC were established and are monitored by the Office of Clinical Research. The MSKCC Data and Safety Monitoring Plans can be found on the MSKCC Intranet at: *http://mskweb2.mskcc.org/irb/index.htm*

There are several different mechanisms by which clinical trials are monitored for data, safety and quality. There are institutional processes in place for quality assurance (e.g., protocol monitoring, compliance and data verification audits, therapeutic response, and staff education on clinical research QA) and departmental procedures for quality control, plus there are two institutional committees that are responsible for monitoring the activities of our clinical trials programs. The committees: *Data and Safety Monitoring Committee (DSMC)* for Phase I and II clinical trials, and the *Data and Safety Monitoring Board (DSMB)* for Phase III clinical trials, report to the Center's Research Council and Institutional Review Board.

During the protocol development and review process, each protocol will be assessed for it's level of risk and degree of monitoring required. Every type of protocol (e.g., NIH sponsored, in-house sponsored, industrial sponsored, NCI cooperative group, etc.) Will be addressed and the monitoring procedures will be established at the time of protocol activation

17.0 PROTECTION OF HUMAN SUBJECTS

The investigator agrees to conduct this study in accordance with the International Conference on Harmonization (ICH) principles of Good Clinical Practice and with the Declaration of Helsinki (1989). The investigator will conduct all aspects of this study in accordance with all national, state, and local laws of the applicable regulatory agencies.

Most patients will be children, adolescents, and young adults because of the nature of these tumors. Patients of both sexes and all ethnic backgrounds are eligible for this study. Alternative treatments are available and will be discussed with patient or legal guardian. Patients are responsible for the costs of physician visits and usual laboratory tests, hospitalizations, and outpatient care. If there is an injury as a result of this research study, emergency care, hospitalization, and outpatient care will be made available by Memorial Hospital and billed to the patient as part of the medical expenses. No money will be provided by Memorial Hospital as compensation for research-related injury.

Patients will not be billed for bevacizumab, but as part of standard care for this medical condition, the patient will be charged for:

- any needed hospitalization
- doctor's care
- any other drugs (including irinotecan and temozolomide) besides bevacizumab needed for treatment.
- laboratory tests
- radiographic studies
- clinic visits

17.1 Privacy

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.

17.2 Serious Adverse Event (SAE) Reporting

Any SAE must be reported to the IRB/PB as soon as possible but no later than 5 calendar days. The IRB/PB requires a Clinical Research Database (CRDB) SAE report be submitted electronically to the SAE Office at sae@mskcc.org containing the following information:

Fields populated from the CRDB:

- Subject's name (generate the report with only initials if it will be sent outside of MSKCC)
- Medical record number
- Disease/histology (if applicable)
- Protocol number and title

Data needing to be entered:

- The date the adverse event occurred
- The adverse event
- Relationship of the adverse event to the treatment (drug, device, or intervention)
- If the AE was expected
- The severity of the AE
- The intervention
- Detailed text that includes the following information:
  - A explanation of how the AE was handled
  - A description of the subject's condition
  - Indication if the subject remains on the study
  - If an amendment will need to be made to the protocol and/or consent form

The PI's signature and the date it was signed are required on the completed report.

All SAE reports must also be forwarded as soon as possible to:

Genentech Drug Safety
Fax: (650) 225-4682 or (650) 225-4683

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

17.2.1 Genentech Adverse Event Reporting Definitions

In the event of an adverse event the first concern will be for the safety of the subject.
A SAE is any sign, symptom or medical condition that emerges during treatment or during a post-treatment follow-up period that (1) was not present at the start of treatment and is not a chronic condition that is part of the patient's medical history, OR (2) was present at the start of 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 (except hospitalization required as part of radiation safety precautions)
- 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.
- Is grade 3 or 4 toxicity excluding myelosuppression.

Assessing Causality:

Investigators are required to assess whether there is a reasonable possibility that bevacizumab and/or irinotecan and/or temozolomide 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 and/or irinotecan and/or temozolomide 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 and/or irinotecan and/or temozolomide administration makes a causal relationship unlikely, or other drugs, therapeutic interventions or underlying conditions provide a sufficient explanation for the observed event.

17.3 Safety Reporting Requirements for IND Exempt Studies

For investigator sponsored IND exempt studies, there are reporting requirements for the FDA in accordance with guidance set forth in 21 CFD 314.80

Postmarketing 15 -Day Alert Report
The Sponsor-Investigator is also 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 at the following address: Central Document Room, 12229 Wilkins Avenue, Rockville, MD 20852.

All Post-marketing 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) 225-4683

For questions related to safety reporting bevacizumab, contact:

Genentech Drug Safety
Tel: 1-888-835-2555
or
Fax: (650) 225-4682 or (650) 225-4683

18.0 INFORMED CONSENT PROCEDURES

Before protocol-specified procedures are carried out, consenting professionals will explain full details of the protocol and study procedures as well as the risks involved to participants prior to their inclusion in the study. Participants will also be informed that they are free to withdraw from the study at any time. All participants must sign an IRB/PB-approved consent form 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.
Each participant and consenting professional will sign the consent form. The participant must receive a copy of the signed informed consent form.

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