TEXAS-OKLAHOMA PEDIATRIC NEURO-ONCOLOGY CONSORTIUM

A PHASE 2 STUDY OF VALPROIC ACID AND RADIATION, FOLLOWED BY MAINTENANCE VALPROIC ACID AND BEVACIZUMAB IN CHILDREN WITH NEWLY DIAGNOSED HIGH-GRADE GLIOMAS OR BRAINSTEM GLIOMAS

STUDY CO-CHAIRS

Jack M. Su, MD, MS
Texas Children's Cancer Center
Baylor College of Medicine
6701 Fannin Street, CC1510
Houston, Texas 77030
Phone: (832) 822-4306
Fax: (832) 825-1503
e-mail: jmsu@txch.org

Susan M. Blaney, MD
Texas Children's Cancer Center
1102 Bates Avenue
Houston, Texas 77030
Phone: (832) 822-1482
Fax: (832) 825-4299
e-mail: sblaney@txch.org

STUDY VICE-CHAIRS

Daniel C. Bowers, MD
Division of Pediatric Hematology-Oncology
UT Southwestern Medical Center at Dallas
5323 Harry Hines Blvd
Dallas, TX 75390-9063
Phone: 214-648-3896
Fax: 214-648-3122

Murali Chintagumpala, MD
Texas Children's Cancer Center
Baylor College of Medicine
6701 Fannin Street, CC1510
Houston, Texas 77030
Phone: (832) 822-4266
Fax: (832) 825-1503
e-mail: mxchinta@txch.org

Research Coordinator
Suzanne H. Wheeler, MPH, RN, CCRP
Texas Children's Cancer Center
1102 Bates Avenue, Suite 1590
Houston, Texas 77030
Phone: (832) 824-4217
Fax: (832) 825-1198
e-mail: shwheele@txch.org

Study Number: TOPNOC_001_08

Protocol Version, Date: Version 3.0, September 27, 2013

TABLE OF CONTENTS

ABSTRACT

Despite recent advances in neurosurgery, radiation, and chemotherapy, children with high-grade gliomas and brainstem gliomas continue to have a dismal prognosis, highlighting the urgent need for novel therapeutic regimens. Valproic acid (VPA), a well-characterized drug used in children with seizures since the 1970's, was recently identified as a histone deacetylase (HDAC) inhibitor and has been shown to have anti-tumor activity in multiple tumor types, including high-grade gliomas. Pre-clinical studies have shown that VPA administered as a single agent inhibits growth of orthotopic xenografts derived from malignant gliomas. In addition, VPA has been shown to enhance the anti-tumor activity of both radiation and chemotherapy. In a recently completed Children's Oncology Group phase 1 study of valproic acid in children with recurrent solid tumors, a partial response was observed in a child with a refractory brainstem glioma and another patient with a refractory glioblastoma multiforme (GBM). Bevacizumab (Avastin), a monoclonal antibody against vascular endothelial growth factor, has also shown promising activity against multiple tumor types, including high-grade gliomas. Two recent studies of the combination of bevacizumab with irinotecan demonstrated an improvement in the 6-month progression free survival (PFS) of adults with recurrent GBM. A subsequent abstract reported at the 2008 American Society of Clinical Oncology Meeting showed that adults randomized to receive bevacizumab alone had statistically identical 6-month PFS versus subjects that received the combination of bevacizumab and irinotecan. As expected, there were fewer adverse events in the bevacizumab alone arm versus the combination arm. We therefore propose a phase 2 trial to evaluate a novel treatment regimen for children with newly diagnosed high grade gliomas that includes administration of daily oral VPA during radiation therapy, followed by i.v. bevacizumab administered every two weeks plus daily oral VPA. The primary aims of this study are: 1) to determine the anti-tumor activity of this regimen as measured by 1- year and 2-year event-free survival (EFS) rate, and 2) to evaluate the toxicities of this regimen. The secondary aims are to characterize several biologic pathways in malignant gliomas, including quantification of DNA repair proteins and markers of tumor angiogenesis, and to correlate these parameters with radiographic response and EFS.

TREATMENT SCHEMA

With the exception of patients with brainstem gliomas, all patients should have the maximal surgical resection that can be safely performed prior to study entry. **Submission of frozen tumor is strongly encouraged. Please coordinate with neurosurgeons and pathologists in advance to ensure that an adequate tumor specimen is obtained (see section 7.0**). After recovery from neurosurgery, all patients will start valproic acid and radiation therapy.

	Pre-XRT	During XRT	Post-XRT	Maintenance Therapy
Day/Wk of Tx	Day -2 to 0	Weeks 1 - 6	Weeks 7 - 10	Weeks 11 - 105
XRT	None	Mon - Fri	*	None
Valproic acid	Daily, oral	Daily, oral	Daily, oral	Daily, oral
Bevacizumab	None	None	None	Every two weeks, iv
Tumor Evaluation	Pre-XRT	None	Week 10	Every 12 wks and as clinically indicated

* XRT may continue into the post-XRT break if there were delays during weeks 1 - 6

Pre-XRT Phase

Valproic acid (VPA) will be started at 15 mg/kg/day divided tid up to 48 hours prior to the initiation of radiation therapy, but no later than the first day of radiation therapy. Patients may begin VPA and radiation therapy as soon as they have adequately recovered from their definitive surgical resection or biopsy, but no later than 30 days after surgery. Specific guidelines for VPA administration are provided in Section 5.2.

During XRT Phase

Radiation should begin within 30 days from surgery or radiographic diagnosis, whichever is the later date. VPA will be continued daily without interruption during radiation therapy. VPA doses will be adjusted in increments of 5 mg/kg/day every 3-5 days to achieve and maintain trough concentrations between 85 to 115 mcg/ml (See Section 5.2.2 and 5.2.3). During this time, patients will receive standard radiation therapy as outlined in Section 9.0.

Post XRT Phase

Patients will continue to receive VPA as during XRT. For patients that required discontinuation of VPA during radiation, they will not resume VPA until start of maintenance therapy. If necessary, patients who had delays in XRT (e.g., secondary to schedule holidays or the need to have a new mask made) will complete their radiotherapy to the total prescribed protocol dose.

Maintenance Therapy

Maintenance therapy will begin 11 weeks after the initiation of radiation therapy. Patients will continue VPA daily during the 4-week break and throughout maintenance therapy. All patients will start bevacizumab, 10 mg/kg iv every two weeks, at the start of maintenance therapy (See Section 5.3). In the absence of unacceptable toxicity or disease progression, patients will continue to receive protocol treatment for a maximum total duration of two years (including the XRT phase).

1.0 GOALS AND OBJECTIVES (SCIENTIFIC AIMS)

1.1 Primary Aims

1.1.1 To determine the efficacy of combining valproic acid (VPA) with radiation, followed by maintenance VPA and bevacizumab in children with newly diagnosed high-grade gliomas and brainstem gliomas, as measured by EFS at one-year and two-years.

1.1.2 To determine the toxicities of VPA when combined with radiation and when combined with bevacizumab in the post-radiotherapy phase.

1.2 Secondary Aims

1.2.1 To quantify proteins related to histone deacetylation (histone H3 and H4 acetylation, HDAC 1 and 2), NHEJ (Ku70/Ku80, DNA-PK) and HRR (Rad51, BRCA1/2) pathway, tumor angiogenesis (VEGF, VEGF receptor 2), and tumor hypoxia (CD31, CA9, hypoxia-inducible factor 2α) by Western analysis if frozen tumors resected at diagnosis and/or at later time points are available.

1.2.2 To perform IHC studies of the molecular markers outlined in 1.2.1 if only paraffin-embedded tumors are available.

1.2.3 To measure NHEJ activity in PBMCs before treatment and after 2 weeks of VPA and radiation.

1.2.4 To measure HDAC2 level and histone acetylation in PBMCs before treatment and after 2 weeks of VPA and radiation.

1.2.5 To quantify MGMT and PARP activity by enzymatic assays in frozen tumors resected at diagnosis and/or at later time points, if available.

1.2.6 To explore potential correlation between the molecular parameters in aims 1.2.1 to 1.2.5 with radiographic response rate and survival outcome.

2.0 BACKGROUND

2.1 High-Grade Gliomas and Brainstem Gliomas in Children

Despite advances in neuro-surgery, radiation therapy, and chemotherapy, children with high-grade gliomas continue to have a dismal prognosis. Although a Children's Cancer Study Group (CCG) trial (CCG-943) showed a promising 5-year EFS of 46% when prednisone, CCNU, and vincristine (PCV) were combined with radiation therapy (1), this result was not duplicated by a subsequent CCG-945 trial. The CCG-945 trial showed that PCV, when combined with radiation therapy, produced a 5-year EFS of 16% and 28% for children with glioblastoma multiforme (GBM) and anaplastic astrocytoma (AA), respectively. Furthermore, a more intensive chemotherapy regimen (eight-drug-in-one regimen) combined with radiation produced nearly identical clinical

outcomes (2, 3). Because of the promising results from a trial of temozolomide and radiation therapy in adults newly diagnosed with GBM (4), a similar trial was conducted through COG (study ACNS0126). The final results of ACNS0126 are pending, but a preliminary analysis showed that children with GBM in this trial had a 1-year EFS of 36%, nearly identical to the 1-year EFS of 32% seen in CCG-945 (5).

Children with brainstem gliomas also have dismal outcomes. A recent review of 22 clinical trials in children with brainstem gliomas showed that only 92 out of 940 children survived, with no evidence that children in recent trials showed any improved EFS or overall survival (OS) compared to those in older series (6). In those children whose tumors were biopsied, the majority (153 out of 229 samples; 67%) were high-grade gliomas (6). Numerous clinical trials involving hyperfractionated radiation (7-10), chemotherapy (8, 10-14) or biologic agents (7, 15, 16), and myeloablative regimens with stem cell rescue (17, 18) uniformly failed to improve EFS or OS compared to historical series. Collectively, these trials showed dismal median EFS (5-8.8 months), median OS (8-12 months), and 2-year survival (5-15%) in children with brainstem gliomas (6).

2.2 Histone Deacetylase (HDAC) and Human Cancers

The lack of improvement in clinical outcome for children with high-grade gliomas and brainstem gliomas from trials conducted in the last two decades highlight the urgent need for novel therapeutic strategies. Recently, HDAC inhibitors have demonstrated promising activity against various human cancers (19, 20). Nucleosomes, the basal unit of chromatin, are composed of DNA coils wrapped around a core of histones with lysine-rich amino terminal tails. The positive charge of the amino tails of histone attracts the negatively charged DNA, and this interaction partly regulates the chromatin structure and gene expression (19). Histone acetyltransferase (HAT) transfers an acetyl moiety from acetyl-CoA to the lysines on the amino tails and neutralizes the positive charge of the histones, leading to decreased affinity for DNA, relaxation of chromatin, and transcriptional activation (19, 21). HDAC removes the acetyl groups and restores the negative charge, leading to chromatin compaction and transcriptional repression (19, 22).

Dysregulation in histone acetylation has been implicated in the development of several human cancers (19, 20), and alterations in the acetylation status of specific lysines have been associated with human cancers as well (23). It is hypothesized that aberrant histone acetylation leads to inappropriate transcriptional repressions and maintenance of the transformed states of human tumors. HDAC inhibitors are hypothesized to reactivate expressions of critical pathways that are abnormally silenced during tumorigenesis, and indeed treatment of multiple human tumor cells with HDAC inhibitors resulted in cell cycle arrest, apoptosis, and differentiation (19, 20).

2.3 Rationale for VPA, an HDAC Inhibitor, in Pediatric High-Grade Gliomas

VPA, an anti-convulsant used in children for over 30 years, was recently found to inhibit HDAC (24). Pre-clinical studies have demonstrated that VPA has *in vitro* and *in vivo* activity against several human cancers (24), and clinical trials of VPA in adult and pediatric malignancies are in progress.

Pre-Clinical Studies of VPA and Other HDAC Inhibitors in CNS Tumors
We have shown that VPA has *in vitro* and *in vivo* activity against medulloblastoma (25, 26). Anti-glioma activity of VPA has also been shown in several studies (24, 27-32). In addition, other HDAC inhibitors, including trichostatin, MS-275, and suberoylanilide hydroxamic acid (SAHA) are also active against malignant glioma (33-36).

Clinical Studies of VPA in Patients with CNS Tumors
In one retrospective analysis of adults with newly diagnosed GBM who underwent radiation therapy followed by chemotherapy, overall survival (OS) was compared amongst patients not taking any anti-convulsant (group A), those taking VPA (group B), or those on enzyme-inducing anti-convulsants (EIAE; group C). The OS of group A and B were 11.6 months versus 13.7 months, and this difference, while not statistically significant, was a noteworthy trend (37). Patients on VPA had a statistically significant improvement in OS (13.7 months) versus those on EIAE (10.8 months, $p = 0.042$).

A COG phase 1 study of VPA (ADVL0419) in children with recurrent/progressive solid tumors, including brain tumors, was recently completed. One out of three children with brainstem gliomas had a partial response (PR) that was sustained for 3 months. Another patient with a GBM (out of two children with high-grade gliomas) had a PR of 5 months duration (Su JM et al, unpublished results). The trough VPA concentrations in these responding patients ranged from 75-110 mcg/ml. In a German trial, a child with a progressive GBM after radiation and chemotherapy achieved a CR for 12 months while maintaining a VPA trough concentration of 160 mcg/ml, but treatment was stopped because of somnolence, and the tumor recurred after drug discontinuation (38). In treatment arm C of the German HIT-GBM trial, 22 children with progressive high-grade gliomas or brainstem gliomas after radiation and chemotherapy were given daily valproic acid as a single agent, and 3 patients have been recently reported as long-term survivors at 4.5, 4.95, and 5 years from time of diagnosis (39).

There appears to be encouraging preliminary clinical data that support further studies of VPA as a novel agent in high-grade gliomas. However, given the non-durable responses observed with VPA as a single agent in children with high-grade gliomas, it is possible that the potential therapeutic value of VPA would be augmented if combined with radiation and/or other drugs. Pre-clinical studies have indeed shown that VPA enhances radiation effect in high-grade gliomas, and additional studies also suggest that VPA inhibits angiogenesis in human cancer models (see discussion below).

VPA and Other HDAC Inhibitors Enhance Radiation Effect
We have shown that VPA enhanced the anti-tumor activity of radiation against two medulloblastoma cell lines by 1.6 to 2.7 fold (Li XN et al, unpublished data). Other investigators have also confirmed that VPA (40-43) and other HDAC inhibitors (44-49) can enhance the radiation effect against various human cancer cell lines, including malignant glioma (40, 41, 44-46, 48) *in vitro*. Camphausen et al. (41) reported that VPA enhanced the *in vivo* radiation response of two subcutaneous xenografts of malignant gliomas, and Entin-Meer et al. (50) showed that another HDAC inhibitor (AN-9)

combined with radiation significantly delayed tumor growth in mice with subcutaneous glioma xenografts.

These studies identified that pre-treatment with VPA or other HDAC inhibitor followed by radiation led to significantly reduced expression of DNA repair proteins such as Ku70, Ku80, and DNA-PK in the non-homologous end-joining (NHEJ) pathway and BRCA1/2 and Rad51 in the homologous recombination repair (HRR) pathways. Since radiation is thought to exert its anti-tumor effect by inducing double-stranded DNA (dsDNA) breaks, and dsDNA breaks are repaired by the NHEJ and HRR pathways, it has been hypothesized that the radiation-enhancement effect of VPA and other HDAC inhibitors are partly mediated by decreased levels of DNA repair proteins. In all the pre-clinical studies examining the radiosensitizing property of VPA and other HDAC inhibitors, optimal radiation enhancement was observed when HDAC inhibition preceded radiation by 24-72 hours and was continued concurrently with radiation.

Anti-angiogenesis Effect of VPA
Our previous studies demonstrated that VPA treatment led to *in vitro* and *in vivo* inhibition of angiogenesis in medulloblastoma (25, 26). Other pre-clinical studies also showed that VPA suppressed angiogenesis in various human cancers by reducing VEGF expression and protein level, inhibiting endothelial cell proliferation, and decreasing neovascularization (51-54). These pre-clinical studies suggest that a combination of VPA and an anti-angiogenesis agent may be a complementary approach in CNS tumors.

VPA Induces Differentiation of Tumor and Stem Cells
Our pre-clinical studies showed that VPA treatment led to neuronal and glial differentiation in medulloblastoma cells (25, 26). Additional studies also showed that VPA promoted differentiation of various neuronal progenitor cells (32, 55-58). The differentiating effect of VPA on other normal or cancerous stem cells has also been reported (59-61), including differentiation of acute myeloid leukemia cells in two clinical trials (62, 63). Since the quiescent cancer stem cell has been hypothesized to be resistant to radiation and chemotherapy, VPA-induced differentiation of the high-grade glioma stem cell may render them more susceptible to treatment.

2.4 Rationale for Bevacizumab in High-Grade Gliomas

Angiogenesis and High-Grade Gliomas
Vascular endothelial growth factor (VEGF) is a potent endothelial cell mitogen and inducer of vascular permeability. VEGF plays a critical role in glioma angiogenesis, as its expression and protein level are elevated in GBM tumor cells and in tumor endothelial cells, but not in the normal brain (64-66). Bevacizumab, a humanized monoclonal IgG1 antibody against the human VEGF-A isoform and its proteolytic fragments, has been shown to decrease vascular permeability and increase apoptosis in intracranial xenografts of human glioblastoma in SCID mice (67).

Bevacizumab, either as a single agent or in combination with other chemotherapy agents, has been extensively studied in multiple clinical trials in more than 8,000 adults with various cancers (68-71). The addition of bevacizumab to various chemotherapy agents

improved the PFS and overall survival (OS) in adults with metastatic colorectal cancer (trial AVF2107g and E3200), non-small cell lung cancer (trial E4599), and metastatic breast cancer (trial E2100; see Bevacizumab Investigator Brochure).

Clinical Trials of Bevacizumab in Adults with Recurrent High-Grade Gliomas
Two recent reports showed promising clinical outcomes in adults with recurrent malignant gliomas after combined bevacizumab and irinotecan treatment. In a phase II study, bevacizumab (10 mg/kg) and irinotecan (125 mg/ m^2 in patients not on EIAE) were given every 2 weeks, and responses were seen in 60% (14 out of 23) of patients with GBM and in 67% (6 out of 9) of patients with AA (72). In a follow up study, in which additional patients with GBM were recruited, and the treatment regimen was modified (bevacizumab 15 mg/kg every 21 days, and irinotecan 125 mg/m^2 weekly for four weeks repeated every 6 weeks), a response rate of 57% was observed (20 out of 35 patients with GBM), and the 6-month PFS was 46% (73). Response rate between the first and second group of patients was nearly identical, suggesting that increased dose-intensity of bevacizumab and/or irinotecan did not significantly improve tumor response. These results represent a significant improvement compared to historical 6-month EFS of < 30% and response rate of < 20% in adults with recurrent GBMs.

In a phase II, randomized, non-comparative clinical trial, Genentech compared the clinical outcome and toxicities of adults with recurrent GBM treated with bevacizumab alone versus those who received bevacizumab and irinotecan (74)(Table 1). The median OS and 6-month PFS were statistically identical in both groups of patients, while those who received bevacizumab alone experienced lower incidences of various toxicities (Table 1). These results are consistent with prior clinical trials of irinotecan, whether as a single agent (75-81) or in combination with other chemotherapy agents (82-84), in adults with recurrent malignant gliomas that showed limited response rates from 0 to 15% and suggested that irinotecan has minimal activity in high-grade gliomas.

Table 1: Efficacy and Toxicities of Bevacizumab Alone Versus Bevacizumab and Irinotecan in Adults with Recurrent GBM

	Bevacizumab (N = 85)	**Bevacizumab and irinotecan (N = 82)**
Efficacy		
Median OS, months	9.2	8.7
6-month PFS, %	42.6	50.3
ORR, %	28.2	37.8
Adverse Events, Grade ≥ 3, %	47.6	67.1
Hemorrhage	0	2.5
Gastrointestinal Perforation	0	1.3
Arterial Thromboembolic Event (ATE)	1.2	2.5
Venous Thromboembolic Event (VTE)	3.6	8.9
Reversible Posterior Leukoencphalopathy Syndrome (RPLS)	0	1.3
Proteinuria	0	1.3
Wound Healing Complication	2.4	1.3

Congestive Heart Failure	0	0
Infection	9.5	13.9
Grade 5 Adverse Events, %	2.4	1.3
Bevacizumab discontinued due to AE, %	3.5	13.9

Clinical Trials of Bevacizumab in Children with Cancers
In a recently completed COG phase I study of bevacizumab in children with recurrent solid tumors (no patients with CNS tumors were included), no DLTs were observed in 18 patients (85) who received up to 15 mg/kg every 2 weeks, and no hemorrhage or thrombosis were seen. Currently, there is an ongoing PBTC trial (PBTC022) of bevacizumab (10 mg/kg every 2 weeks) and irinotecan (125 mg/m^2 every 2 weeks) in children with progressive malignant glioma, medulloblastoma, and ependymoma.

2.5 Rationale for Combining Radiation, VPA, and Bevacizumab

VPA has been used in children since the 1970's, can be taken orally with nearly 100% bioavailability, has a long half-life of 9-20 hours in children, passes through the blood brain barrier effectively (86, 87), and can be given chronically with well-characterized, acceptable toxicities. These biochemical, pharmacokinetic, and clinical properties make VPA an attractive agent for pediatric CNS tumors. Several pre-clinical studies have demonstrated VPA's activity against malignant gliomas, and encouraging responses in children with malignant gliomas and brainstem gliomas were observed in ADVL0419 and HIT-GBM. Additional studies also showed that VPA enhances the effect of radiation against malignant gliomas. Therefore, we hypothesize that combining VPA with up-front radiation is a novel approach worthy of investigation in children with newly diagnosed high-grade gliomas and brainstem gliomas. In both ADVL0419 and HIT-GBM, patients with recurrent tumors who have received prior radiation and chemotherapy tolerated trough VPA concentrations below 110 mcg/ml without DLTs, suggesting that combining VPA with radiation in newly diagnosed patients should be well tolerated. VPA has also been shown to inhibit angiogenesis and promote differentiation of neural progenitor and cancer stem cells. With recent encouraging responses to bevacizumab in adults with recurrent GBM, the combination of the anti-glioma and anti-angiogenic effects of VPA and bevacizumab represents a promising approach in children with high grade and brainstem gliomas. Because VPA and bevacizumab have few overlapping toxicities (see section 3.1 and 3.2), we do not anticipate additive or unexpected toxicities in combining VPA and bevacizumab.

2.6 Proposed VPA Dosing and Targeted Trough Concentration

In HIT-GBM (39), serum trough VPA concentrations in 23 patients averaged 99 mcg/ml (range 52-175 mcg/ml), and no grade 3 or 4 DLTs were reported. Three patients had somnolence, one patient had difficulties in concentration, one patient had thrombocytopenia, and a patient had anemia (all grade 1 or 2). In the COG phase I study, ADVL0419, two patients experienced grade-3 somnolence with trough VPA concentration between 100 and 150 mcg/ml. No DLT was seen in six subsequent patients whose trough concentration was maintained between 70-110 mcg/ml (Su JM et al,

unpublished data). With a starting dose of 15 mg/kg divided tid, all six patients achieved and maintained trough concentration > 75 mcg/ml within the first two weeks.

Based on pre-clinical data demonstrating that the minimal anti-CNS tumor concentration of VPA is > 0.6 mM (~100 mcg/ml), whether as a single agent (25-30) or as an enhancer of radiation activity (40, 41), we propose to maintain VPA trough concentrations as close to 100 mcg/ml as possible. Due to variability in timing of trough sampling and VPA assay, which is estimated to be 10-15%, we propose to maintain VPA trough concentrations between 85-115 mcg/ml (100 +/- 15 mcg/ml) for initial patients.

2.7 Prognostic Markers For Predicting Response and Survival in High-Grade Gliomas

In adults with newly diagnosed GBM receiving temozolomide and radiation, it was shown that those with methylated promoter of the MGMT (O^6-methylguanine-DNA methyltransferase) DNA-repair gene in their tumors had improved clinical outcomes compared to those with absence of MGMT promoter methylation (88). Such a correlation of MGMT promoter methylation and/or MGMT activity with improved clinical outcome in children with high-grade gliomas has not been confirmed yet.

In the two clinical trials of bevacizumab and irinotecan in adults with progressive malignant gliomas (72, 73), semi-quantitative immunohistochemistry (IHC) studies of VEGF, VEGF receptor 2, CD31 (surrogate marker for vascularity), hypoxia-inducible carbonic anydrase 9 (CA9), and hypoxia-inducible factor-2a were performed in 45 tumor specimens. High VEGF level was associated with increased likelihood of radiographic response, and high CA9 level was associated with poor survival outcome (89). Since we will be incorporating bevacizumab into the proposed maintenance chemotherapy, these molecular parameters should also be studied and correlated with clinical outcome.

Because several pre-clinical studies have documented that VPA enhancement of radiation effect was associated with decreased levels of DNA repair proteins in the NHEJ and HRR pathways, we will also determine the levels of these DNA repair proteins in the untreated tumors and correlate them with clinical outcome. We will also quantify NHEJ activity in peripheral blood mononuclear cells (PBMC) before and after 2 weeks of VPA and radiation to measure VPA's potential inhibition of double-stranded DNA break repair. In addition, we also propose to study pre-treatment poly(ADP-ribose) polymerase (PARP) activity in pediatric high-grade gliomas, since PARP is a critical enzyme for activating both single stranded and double-stranded DNA break repair (90).

Several pre-clinical studies have shown that inhibition of HDAC2 appears to be critical for various aspects of HDAC inhibitors' anti-tumor effect, including chromatin decondensation and remodeling, transcriptional activation, and induced histone acetylation (91-94). Subsequent clinical trials have confirmed that baseline expression of HDAC2 in PBMCs (as surrogates for tumor specimens) correlated directly with increases in histone H3 and H4 acetylation after HDAC inhibitor treatment (92, 95-97). Therefore, baseline and post-treatment HDAC2 level in PBMCs may be an additional surrogate marker for measuring VPA's biologic effect in tumors.

2.8 Rationale for Amending Eligibility Criterion Regarding Intra-Tumoral Hemorrhage

Because of bevacizumab's mechanism of action, intra-tumoral and/or intracranial hemorrhage was a potential concern with earlier clinical trials. With available published data, it is reassuring that the rate of intra-tumoral/intracranial hemorrhage is quite low, and revising or current eligibility criteria to include patients with asymptomatic intra-tumoral hemorrhage, in a patient population that has a significant incidence of intra-tumoral hemorrhage at the time of diagnosis, prior to initiation of any surgery or other treatment, with few effective treatments available, is a reasonable option. Final data from the phase II clinical trials of 163 adults with recurrent GBM receiving bevacizumab, either alone or in combination with irinotecan, showed a rate of intracranial hemorrhage of 2.4 (bevacizumab alone) and 3.8% (bevacizumab and irinotecan); patients with stable intra-tumoral/intracranial hemorrhage, regardless of size of hemorrhage, were eligible for this trial (98). A follow up clinical trial, in which 125 adults with newly diagnosed GBM received bevacizumab, temozolomide, and radiation immediately after initial tumor resection, included patients with grade 1 (asymptomatic) intracranial hemorrhage prior to study entry and showed rate of intracranial hemorrhage to be less than 1% (1 out of 125 patients) (99). In a recent pediatric phase II clinical trial, 31 children with either high-grade glioma or DIPG received bevacizumab and irinotecan, and patients with pre-existing and stable intra-tumoral hemorrhage were eligible for study participation; 4 patients experienced asymptomatic grade 1 CNS hemorrhage. The currently open Children's Oncology Group phase II clinical trial for high-grade gliomas (ACNS0822), which includes bevacizumab during and after radiation therapy, also allow patients with pre-existing and stable CNS hemorrhage to participate in the trial.

Of the twenty seven children who have been enrolled onto our clinical trial, two patients experienced grade-1 CNS hemorrhage and another patient had a grade-2 hemorrhage, all occurring at the time of tumor progression. In a patient population with dismal prognosis and few effective treatment options, we feel that, in light of the published adult and pediatric data and our trial experience to date, it is reasonably safe to include patients with **asymptomatic, stable** intra-tumoral or intracranial hemorrhage $\leq$ 1 cm in the widest dimension, and the eligibility guideline has been accordingly revised in section 4.1.6.

2.9 Addition of a Participate Site to TOPNOC

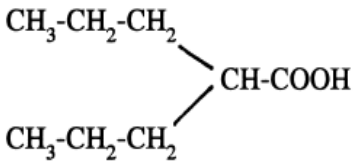
Dr. Ben Carcamo will serve as the PI of University of Texas, El Paso, which will be added as a member of the consortium.

3.0 AGENT INFORMATION

3.1 Valproic Acid

Depakene (syrup and capsule), Depakote (capsule and sprinkles), or other generic formulations of valproic acid are permissible.

3.1.1 Structure and molecular weight: 2-propylpentanoic acid (NSC # 93819), molecular weight 144.21



3.1.2 Supplier
Commercially available

3.1.3 Formulation
The agent is supplied either as syrup (250 mg/5 ml) or as a capsule (125 or 250 mg capsules).

3.1.4 Storage
The capsules should be stored at 15-25°C, and the syrup should be kept at below 25°C until the time of use.

3.1.5 Solution Preparation
No preparation is necessary prior to administration.

3.1.6 Stability
The syrup and capsules are stable until the manufacturer's suggested date of expiration.

3.1.7 Administration
The capsules can be swallowed whole without breakage, or alternatively, the content of the capsule can be sprinkled and mixed with water, juice, or food. Syrup can be mixed with water, juices, or food.

3.1.8 Drug Interactions
VPA has documented interactions with multiple anti-epileptics, and some of the severe toxicities of VPA, such as fatal hepatitis or hyperammonemia encephalopathy (see section 5.6.8 and 5.6.9), are associated with young children (age < 24 months) taking multiple anti-convulsants. Therefore, the use of other anti-epileptics should be avoided.

Other drugs with documented effects on VPA metabolism include acyclovir, aspirin and other salilcylates, carbapenem antibiotics (imipenem, meropenem), cholestyramine, chlorpromazine, isoniazid, macrolide antibiotics (clarithromycin, azithromycin, erythromycin), and rifampin. **If any of these drugs need to be administered after a patient has achieved the targeted VPA concentration, then twice weekly VPA trough concentration, CBC, and liver function tests should be monitored for at least 2 consecutive weeks (longer if clinically indicated).**

VPA has documented effects on the metabolism of amitriptyline, nortriptyline, clonazepam, diazepam, nimodipine, tolbutamide, tricyclic anti-depressants,

warfarin, and zidovudine. Patients on protocol therapy who are also taking those medications should be monitored closely for toxicities and/or therapeutic effects of those medications.

3.1.9 Toxicities

The most common side effects are gastrointestinal (nausea, vomiting, diarrhea), dermatological (dry skin, rash), and neurological (somnolence, ataxia, blurred vision, emotional lability) in nature, which seldom require discontinuation of treatment. See Table 2 for a complete listing of known VPA toxicities.

Table 2: Known Toxicities of Valproic Acid in Children

	Common (21-100% Frequency)	**Occasional (5-20% Frequency)**	**Rare (< 5% Frequency)**
Immediate: Within 1-2 days of receiving drug	Nausea, vomiting, abdominal pain, diarrhea, headache	Anorexia, dyspepsia, flu-like syndromes	
Prompt: Within 2-3 weeks, prior to next cycle.	Somnolence, fatigue, tremor, asthenia, dizziness, alopecia, rash, dry skin, pruritis, paresthesia	Diplopia, blurred vision, ataxia, nystagmus, headache, amnesia, insomnia, emotional lability, anxiety, tinnitus, weight gain or loss	Stomatitis, gingivitis, confusion, hyperactivity, coma, erythema multiforme, Steven-Johnson, toxic epidermal necrolysis
Delayed: Anytime after above.		Hypothyroidism, thrombocytopenia	Anemia, neutropenia, lymphopenia, leukopenia, SIADH, periodontal abscess, amenorrhea, menstrual irregularities, urinary frequency, vaginitis, tachycardia, arrhythmia, hypertension, hearing loss, interstitial nephritis, pneumonia
Late: Anytime after completion of treatment			Tetratogenic effects on newborns
Unknown Timing and Frequency			Pancreatitis, hepatitis,

			hyperammonemia, encephalopathy, bone marrow aplasia or myelodysplastic syndrome, abnormal coagulation tests and von Willebrand panel, bleeding, neural tube defects to fetuses

CNS toxicities

Neurological symptoms such as ataxia, diplopia, somnolence, fatigue, dizziness, emotional lability, or difficulty with memory or concentration are almost always transient and resolve after temporarily discontinuing VPA or dose reduction. In ADVL0419, of the 20 children who maintained trough VPA concentration between 100-150 mcg/ml, two patients had grade 3 somnolence, which resolved in 48 hours after VPA was discontinued. Six subsequent patients received VPA to maintain trough concentration of 75-100 mcg/ml, and none experienced $\geq$ grade 3 CNS toxicities. In the HIT-GBM-C treatment arm (39), of the 44 children who maintained VPA concentration between 52-175 mcg/ml, three patients experienced grade 1 or 2 somnolence, and one patient had grade 1 or 2 difficulty in concentration, but there was no apparent correlation between trough concentration and CNS toxicities.

Hematologic toxicities

Thrombocytopenia in children receiving VPA is generally transient and mild in severity, resolves with dose reduction, and is most commonly associated with serum trough > 140 mcg/ml (100, 101). Two large retrospective analyses showed that severe thrombocytopenia, defined as platelet count < 50,000/mm^3, was rarely observed at serum concentrations < 150 mcg/ml (102, 103). There was no specific association between severity of thrombocytopenia and duration of treatment. Thrombocytopenia most commonly resolves despite continuation of VPA at lower doses (104, 105). In ADVL0419, only one patient experienced grade-4 thrombocytopenia, which resolved after reduction of the VPA dose to achieve a trough concentration < 100 mcg/ml. Seven instances of transient grade-1 and two of grade-2 thrombocytopenia occurred and all resolved without requiring drug discontinuation or dose reduction. In the HIT-GBM trial, only one patient had grade 2 thrombocytopenia.

Anemia, neutropenia, and lymphopenia are less common than thrombocytopenia in children receiving VPA, occurring in < 5% of patients. In ADVL0419, one grade 4 anemia, one grade 4 lymphopenia, and one grade 3 neutropenia were reported, and no febrile neutropenia or bacteremia were documented. In HIT-GBM-C, only one grade 2 anemia was reported.

In summary, the myelosuppression of VPA is generally self-limited and well tolerated. However, rare cases of bone marrow aplasia and myelodysplastic syndrome have been previously reported in children on VPA (100).

Hepatic encephalopathy and hyperammonemia
One of the most severe toxicity of VPA is fatal hepatic failure, estimated at an incidence of one in 33,000 adults and children (86, 106). The syndrome is characterized by elevation in transaminase and bilirubin, decreased fibrinogen, with or without hyperammonemia, followed by rapid neurological deterioration, encephalopathy, and coma. Although there is no clear association with dose, duration, or serum drug concentration, children younger than 2 years of age and taking multiple anti-convulsants have been identified as the group with the highest risk, with an estimated incidence of 1:600 (106) to 1: 5000 (107). Therefore, we will be excluding children younger than 2 years of age and strongly discourage the use or other concomitant anti-convulsants.

Hyperammonemia has been reported in association with VPA despite normal liver function tests, and in patients with unexplained lethargy, vomiting, and/or changes in mental status, hyperammonemic encephalopathy should be considered (108). Although there is no association with duration of treatment or serum VPA concentration, identified risk factors include multiple anti-convulsants, carnitine deficiency, and urea cycle or other metabolic disorders. Discontinuation of VPA and supportive care, including carnitine supplementation, are the recommended treatments (109, 110).

Pancreatitis
Another potentially fatal toxicity of VPA is pancreatitis. The incidence of pancreatitis in children taking VPA is unknown; a recent retrospective review in the English literature of all 33 cases of pancreatitis in children taking VPA identified 9 fatal cases, with no association between pancreatitis and the dose or serum concentration of VPA (111). We will exclude any patient with a prior history of pancreatitis and/or abnormal lipase/amylase levels from this trial.

Tetratogenicity
There is an estimated 1-2% incidence of neural tube defects in babies of mother taking VPA (86), so all female patients of child-bearing age should have a negative urine pregnancy test prior to enrollment.

3.2 Bevacizumab (Avastin™, rhuMAb VEGF)

3.2.1 Description and molecular weight

Bevacizumab is a recombinant humanized monoclonal anti-VEGF antibody, consisting of 97% human and 3% of murine amino acid sequences. The agent is composed of human IgG framework and murine antigen-binding complenmentarity-determining regions. Bevacizumab blocks the binding of

VEGF to its receptor, resulting in inhibition of angiogenesis. Bevacizumab's molecular weight is approximately 149,000 daltons.

3.2.2. Supplied by

Genentech Inc. will provide the drug to Texas Children's Cancer Center who will then distribute to each site. Please see Appendix IA for instructions to place a drug order. The pharmacy must be notified at least 5 working days in advance of the anticipated date of use.

The bevacizumab to be supplied for this protocol is intended for patients enrolled on this clinical trial use only. Commercially available Avastin™ should not be utilized in its place.

3.2.3 Formulation

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

3.2.4 Storage and stability

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.

3.2.5 Route and method of administration

The calculated dose of bevacizumab should be diluted in a total volume of 100 mL of 0.9% Sodium Chloride Injection, USP. Administration will be as a continuous IV infusion. Anaphylaxis precautions should be observed during study drug administration.

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

Immediately after completion of the infusion, flush the IV infusion line with 30 mL of 0.9% sodium chloride at the same rate as the bevacizumab infusion.

3.2.6 Bevacizumab-specific toxicities

The major adverse events associated with bevacizumab are listed below. Please refer to the Investigator Brochure for detailed description of all toxicities.

3.2.6.1 Infusion-related reactions

Infusion reactions with bevacizumab are uncommon (< 3%) and rarely severe (0.2%). Infusion reactions may include rash, urticaria, fever, rigors, hypertension, hypotension, wheezing, hypoxia, or bronchospasm. Anaphylaxis precautions should be observed during study drug administration.

3.2.6.2 Hypertension

An increased incidence of hypertension has been observed in adults treated with bevacizumab, with an estimated incidence of 20-30% across trials. 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) (112, 113). Hypertension requiring treatment was not observed in children enrolled on the initial pediatric phase I trial of bevacizumab (85).

Monitoring of blood pressure is recommended during bevacizumab therapy. Optimal control of blood pressure according to standard public health guidelines is recommended. Temporary interruption of bevacizumab therapy is recommended in patients with hypertension requiring medical therapy until adequate control is achieved.
If hypertension cannot be controlled with medical therapy, bevacizumab therapy should be permanently discontinued.
Bevacizumab should be permanently discontinued in patients who develop hypertensive crisis or hypertensive encephalopathy.

3.2.6.3 Proteinuria

An increased incidence of proteinuria has been observed in patients treated with bevacizumab, with up to 38% of patients experiencing proteinuria. 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. In adults with recurrent GBMs, the incidence of $\geq$ grade 3 proteinuria was 1.3% in those who received bevacizumab and irinotecan, compared to 0% in patients who received bevacizumab alone (74). Patients with a history of

hypertension may be at increased risk for the development of proteinuria when treated with bevacizumab.

3.2.6.4 Venous thromboembolism (VTE)

In clinical trials across all indications the overall incidence of VTE events was 2.8% to 17.3% in the bevacizumab-containing arms compared with 3.2% to 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.

In adults with recurrent GBMs, the incidence of ≥ grade 3 VTE was 8.9% in those who received bevacizumab and irinotecan, compared to 3.6% in patients who received bevacizumab alone (74). None of the children who received bevacizumab in the COG phase 1 trial (85) experienced any venous thrombotic complications.

3.2.6.5 Arterial thromboembolic events (ATE)

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

In adults with recurrent GBMs, the incidence of ≥ grade 3 ATE was 2.5% in those who received bevacizumab and irinotecan, compared to 1.2% in patients who received bevacizumab alone (74). None of the children who received bevacizumab in the COG phase 1 trial (85) experienced any arterial thrombotic complications.

3.2.6.6 Gastrointestinal perforation

Patients with metastatic carcinoma may be at increased risk for the development of gastrointestinal perforation and fistula when treated with bevacizumab and chemotherapy. A causal association of intra-

abdominal inflammatory processes and gastrointestinal perforation to bevacizumab treatment has not been established. Nevertheless, caution should be exercised when treating patients with intra-abdominal inflammatory processes with bevacizumab. Gastrointestinal perforation has been reported in other trials in non-colorectal cancer populations (e.g., ovarian, renal cell, pancreas, breast, and NSCLC) and may be higher in incidence in some tumor types.

In adults with recurrent GBMs, the incidence of ≥ grade 3 gastrointestinal perforation was 1.3% in those who received bevacizumab and irinotecan, compared to 0% in patients who received bevacizumab alone (74).

3.2.6.7 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.

3.2.6.8 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. Surgery in patients currently receiving bevacizumab is not recommended. No definitive data are available to define a safe interval after bevacizumab exposure with respect to wound healing risk in patients receiving elective surgery; however, the estimated half life of bevacizumab is 21 days.

In adults with recurrent GBMs, the incidence of ≥ grade 3 wound healing complication was 1.3% in those who received bevacizumab and irinotecan, compared to 2.4% in patients who received bevacizumab alone (74).

3.2.6.9 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. 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. GI hemorrhages, including rectal bleeding and melena have been reported in patients with CRC, and have been assessed as tumor-associated hemorrhages. Tumor-associated hemorrhages were also seen rarely in other tumor types and locations, including a case of CNS bleeding in a patient with hepatoma with occult CNS metastases and a patient who developed continuous oozing of blood from a thigh sarcoma with necrosis.

In adults with recurrent GBMs, the incidence of $\geq$ grade 3 hemorrhage was 2.5% in those who received bevacizumab and irinotecan, compared to 0% in patients who received bevacizumab alone (74). None of the children who received bevacizumab in the COG phase 1 trial (85) experienced any tumor-associated hemorrhage.

*Mucocutaneous Hemorrhage***:** Across all bevacizumab clinical trials, mucocutaneous hemorrhage has been seen in 20%-40% of patients treated with bevacizumab. These were most commonly NCI-CTC Grade 1 epistaxis that lasted less than 5 minutes, resolved without medical intervention and did not require any changes in bevacizumab treatment regimen. There have also been less common events of minor mucocutaneous hemorrhage in other locations, such as gingival bleeding and vaginal bleeding.

3.2.6.10 Reversible Posterior Leukoencephalopathy Syndrome (RLPS)

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. In adults with recurrent GBMs, the incidence of RPLS

was 1.3% in those who received bevacizumab and irinotecan, compared to 0% in patients who received bevacizumab alone (74). None of the children who received bevacizumab in the COG phase 1 trial (85) experienced RLPS.

3.2.6.11 Congestive heart failure (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 (114).

None of the adults with recurrent GBMs treated with bevacizumab +/- irinotecan experienced CHF (74). None of the children who received bevacizumab in the COG phase 1 trial (85) experienced CHF.

3.2.6.12 Neutropenia: Increased rates of severe neutropenia, febrile neutropenia, or infection with severe neutropenia (including some fatalities) have been observed in patients treated with myelotoxic chemotherapy regimens plus bevacizumab in comparison to chemotherapy alone (115). In adults with recurrent GBMs, the incidence of $\geq$ grade 3 infection was 13.9% in those who received bevacizumab and irinotecan, compared to 9.5% in patients who received bevacizumab alone (74). Neutropenia was not a dose-limiting toxicity in those children who received bevacizumab in the COG phase 1 trial (85).

3.2.6.13 Bone defects in growing children: The growth plates of actively growing bones consist of avascular cartilage that is replaced by bone. This is mediated by ingrowth of metaphyseal blood vessels that is directed by a VEGF gradient. Studies in mice indicate that trabecular bone formation can be suppressed by VEGF inhibitors (116). Similarly, VEGF inhibitors caused metaphyseal dysplasia (hypertrophied chondrocytes and loss of metaphyseal capillary invasion) only in monkeys with open growth plates that was reversible on cessation of treatment (117). Although large studies of the effects of bevacizumab on bone growth in humans are unavailable, a recent case report seems to suggest that bevacizumab was possibly causative in the occurrence of metaphyseal lytic lesions in a child with viscero-cutaneous

angiomatosis (118). There was complete healing of these lesions following cessation of bevacizumab (118).

3.2.7 Bevacizumab Inventory Records
The investigator, or a responsible party designated by the investigator, must maintain a careful record of the inventory and disposition of bevacizumab received and administered during protocol therapy. Please complete Appendix IB and fax to Sue Burlingame (Phone: 832-824-1532; Fax: 832-825-1198) or Monica Rodriguez (Phone: 832-824-1531; Fax 832-825-2005) every 12 weeks during maintenance therapy.

4.0 PATIENT ELIGIBILITY

All clinical and laboratory studies to determine eligibility must be performed within 7 days prior to enrollment unless otherwise indicated. If more than 7 calendar days elapse between the date eligibility studies outlined in Section 6.0 were obtained and the start date of treatment, then the following studies must be repeated prior to treatment: CBC with differential, bilirubin, ALT (SGPT) and serum creatinine. If any of these repeat laboratory studies are outside the parameters required for eligibility (labs may again be repeated within 48-72 hours), then the patient will not be eligible for protocol therapy. Imaging studies are required within 2 weeks prior to start of protocol therapy.

Important note: The eligibility criteria listed below are interpreted literally. All clinical and laboratory data required for determining eligibility of a patient enrolled on this trial must be available in the patient's medical or research record which will serve as the source document for verification at the time of audit.

4.1 Inclusion Criteria

4.1.1 Age: Patient must be $\geq$ 3 years and $\leq$ 21 years of age at the time of study enrollment.

4.1.2 Diagnosis: Patients must have histologic verifications of a glioblastoma multiforme, anaplastic astrocytoma, gliomatosis cerebri (WHO grade III or IV glioma with diffuse parenchymal and/or leptomeningeal involvement), or gliosarcoma at the time of study enrollment.

Patients with newly diagnosed intrinsic brainstem gliomas, defined as tumors with a pontine epicenter and diffuse rather than focal involvement of the pons, with or without extension to adjacent medulla or midbrain, are eligible without histologic confirmation. Patients with brainstem tumors that do not meet these criteria or not considered to be typical intrinsic pontine gliomas will only be eligible if the tumors are biopsied and proven to be a grade III or IV glioma (anaplastic astrocytoma, glioblastoma multiforme, gliosarcoma).

4.1.3 Performance Level: Patients must have Karnofsky Performance Score (for patients > 16 years of age) or Lansky Performance Score (for patients ≤ 16 years of age) ≥ 50% assessed within two weeks of study enrollment. Patients who are unable to walk because of paralysis, but who are up in a wheelchair, will be considered ambulatory for the purpose of assessing the performance score.

4.1.4 Prior Therapy: Patients must not have received any prior chemotherapy, radiation therapy, biologic therapy, or bone marrow transplant. Surgery and dexamethasone are permitted prior to study entry. In patients who require anti-convulsants prior to study entry, it is permissible to start VPA, but trough VPA concentration must be repeated within 48 hours of study entry.

4.1.5 Organ Function Requirements:

4.1.5.1 Adequate bone marrow function defined as:

a. Hgb ≥ 8 gm/dL (transfusion independent)
b. Platelet count ≥ 100,000/mm^3 (transfusion independent)
c. Absolute neutrophil count (ANC) ≥ 1,000/ mm^3

4.1.5.2 Adequate liver function defined as:

a. Bilirubin (sum of conjugated + unconjugated) ≤ 1.5 times institutional upper limit of normal (ULN) for age**.**
b. SGPT (ALT) ≤ 2.5 times institutional ULN for age.
c. Serum albumin ≥ 2 g/dL.

4.1.5.3 Adequate renal function defined as:

a. Urine protein (albumin)/creatinine ratio of < 1.0 (See Appendix II)
b. Creatinine clearance or radioisotope GFR ≥ 70 ml/min/1.73m^2 OR
c. A serum creatinine based on age and gender as follow:

Age	**Maximum Serum Creatinine (mg/dL)**	
	Male	**Female**
2 to < 6 years	0.8	0.8
6 to < 10 years	1	1
10 to < 13 years	1.2	1.2
13 to < 16 years	1.5	1.4
≥ 16 years	1.7	1.4

The threshold creatinine values in this table were derived from the Schwartz formula for estimating GFR (Schwartz et al. J. Peds, 106:522, 1985) utilizing child length and stature data published by the CDC.

4.1.5.4 Amylase and Lipase: Amylase and lipase ≤ 2 times institutional ULN for age.

4.1.5.5 Coagulation: Patients must **NOT** have a prolonged PT or PTT (greater than 1.2 times the institutional upper limit of normal**), AND** the INR must be < 1.5.

4.1.6 CNS Hemorrhage: **MRI ECHO gradient sequences are required** to evaluate for the presence or absence of CNS hemorrhage. Patients with intra-tumoral and/or CNS hemorrhage are eligible for study entry **if they fulfill the following guideline**:

- Patients with an asymptomatic intra-tumoral/intracranial hemorrhage measuring ≤ 1 cm in the widest dimension on MRI, at the time of diagnosis, after surgery, and/or any time prior to study enrollment, are eligible; hemorrhage **must not have progressed** on MRI prior to initiation of protocol therapy; patients **must not have developed progressive symptoms thought to be related** to the intra-tumoral/intracranial hemorrhage prior to initiation of protocol therapy
- patients with > 1 asymptomatic intra-tumoral/intracranial hemorrhage but all measuring ≤ 1 cm in the widest dimension on MRI are eligible if they fulfill the guidelines described above
- Patients with asymptomatic post-operative hemorrhage in and/or around the surgical cavity are eligible for study entry if they otherwise fulfill the guidelines described above.
- Patients with an intra-tumoral hemorrhage > 1 cm at diagnosis but who demonstrates minimal post-operative hemorrhage as described above after tumor resection **are eligible for study**

4.1.7 Surgery: Patients must begin radiation therapy within 30 days of surgery or radiographic diagnosis, whichever is the later date. Date of surgery or radiographic diagnosis is considered day 1 (radiation treatment must start no later than day 31). If a patient has a biopsy followed by a surgical resection then the date of the surgical resection is considered day 1.

4.1.8 Informed Consent: All patients and/or their legal guardians must sign a written informed consent. Assent, when appropriate, will be obtained according to institutional guidelines.

4.1.9 Patient Registration: Treating physicians or designees should contact the Study Chair to confirm the availability of study slots prior to consenting patients/families. Patients' on-study registration must be confirmed before starting protocol therapy. Please contact Suzanne Wheeler (Phone: 832-824-4217; Fax: 832-825-1198) or Sue Burlingame (Phone: 832-824-1532; Fax: 832-825-1198) to register a patient. A signed eligibility checklist and the signed informed consent form along with supporting source documents (pathology report, MRI reports, laboratory studies, etc.) must be faxed at the time of study registration.

4.2 Exclusion Criteria

4.2.1 Pregnancy or Breast-Feeding: Females of reproductive potential must not be pregnant or lactating. Males or females of reproductive potential may not participate unless they have agreed to use an effective contraceptive method.

4.2.2 Cardiac Function: Patients with active or history of cardiac (CHF, myocardial infarction, myocarditis) disease are excluded from this trial.

4.2.3 Concomitant Medications: Patients receiving any of the following medications are not eligible for study:

a. Anti-cancer therapy or investigational agents
b. Anti-coagulants (except for heparin to maintain the patency of central venous catheters).
c. Growth factors for white blood cell, red blood cell or platelet support
d. Aspirin (> 81 mg/day)
e. Non-steroidal anti-inflammatory drugs
f. Clopidogrel (Plavix), dipyridamole (Persantine), or any other drug that inhibits platelet functions
g. Anti-convulsants: patients on any anti-convulsant with the exception of VPA are eligible for study entry. It is **STRONGLY RECCOMMENED** that a neurology consult be obtained to enable discontinuation of all anti-convulsant other than VPA, whenever possible.

4.2.4 Infection: Patients who have an uncontrolled infection are not eligible.

4.2.5 Hypertension:

- Patients with inadequately controlled systemic hypertension (SBP and/or DBP > 95th percentile for age and height; see Appendix III)
- Patients with a prior history of hypertensive crisis and/or hypertensive encephalopathy

If a BP measurement prior to registration is > 95th percentile for age and height, it must be rechecked and documented to be < 95th percentile for age and height prior to registration. If a patient falls between the height or weight percentiles, site should average the value as appropriate. For patients ≥ 18 years, use adult normal ranges for blood pressure. Patients with hypertension are eligible if their blood pressures become < 95th percentile after anti-hypertensive medications.

4.2.6 Prior Ischemic Events: Patients with a history of stroke, myocardial infarction, or unstable angina within 6 months prior to registration are not eligible.

4.2.7 Vascular Disease: Patients with significant vascular disease (e.g., aortic aneurysm requiring surgical repair or recent peripheral arterial thrombosis) within 6 months prior to registration will not be eligible.

4.2.8 Bleeding /Coagulopathy: Patients with a history of hemoptysis, bleeding diathesis, known platelet disorder, or coagulopathy are not eligible.

4.2.9 Gastrointestinal: Patients with a history of abdominal fistula or GI perforation within 6 months prior to registration are not eligible.

4.2.10 Urea Cycle Disorder: Patients with a known or suspected urea cycle or other metabolic disorder are not eligible.

4.2.11 Metaphyseal Plate Abnormality: Patients with abnormality of the tibial metaphyseal plate on plain X-ray prior to study entry are not eligible.

4.2.12 Patients with a history of a serious non-healing wound, ulcer, or bone fracture are not eligible.

4.2.13 Patients with any clinically significant systemic illness, including serious infection, pulmonary, hepatic, or other organ impairment, that would compromise tolerance and/or timely completion of protocol therapy.

4.2.14 Patients who, in the opinion of the investigator, may not be able to comply with the safety monitoring requirements and/or follow-up studies of this trial.

4.2.15 Patients with a known hypersensitivity to any component of bevacizumab are not eligible for this trial.

5.0 TREATMENT PLAN AND DOSE MODIFICATION

5.1 Treatment Plan

With the exception of patients with brainstem gliomas, all patients should have the maximal surgical resection that can be safely performed prior to study entry. **Submission of frozen tumor for biology studies is strongly encouraged. Please coordinate with neurosurgeons and pathologists in advance to ensure that an adequate tumor specimen is obtained (See section 7.0)**. After recovery from neurosurgery, all patients will start valproic acid and radiation therapy.

	Pre-XRT	During XRT	Post-XRT	Maintenance Therapy
Day/Wk of Tx	Day -2 to 0	Weeks 1 - 6	Weeks 7 - 10	Weeks 11 - 105
XRT	None	Mon - Fri	*	None
Valproic acid	Daily, oral	Daily, oral	Daily, oral	Daily, oral
Bevacizumab	None	None	None	Every two weeks, iv
Tumor Evaluations	Pre-XRT	None	Week 10	Every 12 wks and as clinically indicated

* XRT may continue into the Break if there were delays during weeks 1 - 6

5.1.1 Pre-Radiation Phase

VPA will be started at 15 mg/kg/day divided tid, ideally 48 hours prior to first day of radiation therapy, but no later than the first day of radiation therapy. Patients may also begin VPA sooner if they have post-operative seizures and

require an anti-convulsant. Guidelines for VPA administration are provided in Section 5.2.

5.1.2 Radiation Phase

Radiation therapy should begin within 30 days of definitive surgery or radiographic diagnosis, whichever is the later date. Date of surgery or radiographic diagnosis is considered day 1, and radiation should start no later than day 31. VPA will be continued daily without interruption during radiation therapy. VPA doses will be adjusted in increments of 5 mg/kg/day every 3-5 days to achieve and maintain trough concentrations between 85 to 115 mcg/ml (See Section 5.2.2 and 5.2.3). During this time patients will receive standard radiation therapy as outlined in Section 9.0.

5.1.3 Post Radiation Phase

Patients will continue to receive VPA as during XRT. If necessary, patients who had delays in XRT (e.g., secondary to schedule holidays or the need to have a new mask made) will complete their radiotherapy to the total prescribed protocol dose (See Section 9.0).

5.1.4 Maintenance Phase

Maintenance therapy will begin 4 weeks after completion of radiation or week 11, whichever comes first. Patients with new hemorrhage on MRI will not be eligible for starting maintenance therapy, with the exception of asymptomatic punctate hemorrhage. Patients with improving or resolved pre-existing hemorrhage will be permitted to start maintenance treatment.

Patients will continue VPA daily during maintenance therapy. All patients will start bevacizumab, 10 mg/kg iv every two weeks, at the start of maintenance therapy (See Section 5.3). Maintenance therapy will continue uninterrupted if all laboratory tests as outlined in section 6.0 continue to meet on-study criteria as defined in Section 4.1.5. In the absence of unacceptable toxicity or disease progression, patients will continue to receive protocol treatment for a maximum total duration of two years (including the XRT phase).

5.2 Valproic Acid (VPA) Administration and Monitoring Guidelines

5.2.1 Drug Administration

Valproic acid will be administered daily in three divided doses. There will be no planned dosing interruptions. Dose modifications for toxicity are outlined in Section 5.4.

5.2.2 Starting Dose and Dose Escalations

The starting dose of VPA will be 15 mg/kg/day, divided tid. Trough VPA concentration (measured 30 to 60 minutes before the next dose) will be measured every 3 to 5 days (if day 3 falls on a weekend) to reach a target trough VPA concentration of 100 mcg/mL (range, 85-115 mcg/ml). The VPA dose will

be adjusted in increments of 5 mg/kg/day to reach the desired trough concentration. Patients who initially reached the desired VPA concentration range but then required subsequent dose escation(s) to maintain targeted trough concentration should also have trough VPA, CBC, and liver functions measured every 3-5 days until target is reached. **Adjustment of VPA dose MUST be calculated based on the most recent weight.**

If available, a liquid/syrup preparation of valproic acid is preferred, and the dose should be rounded to the nearest 5 mg. Doses with Depakote sprinkles or capsule should be rounded down to the nearest 125 mg (unit dose for sprinkles or capsule). **Use of extended release tablets of valproic acid is not permitted.**

Patients/families should attempt to adhere to a strict q 8 hour administration schedule (e.g. 7 am - 3 pm -11 pm schedule) as much as possible. Medication diaries (see Appendix IV) are required to document compliance. During radiation therapy, medication diaries should be checked and faxed weekly. After radiation therapy, medication diaries should be checked every 2 weeks and faxed every 4 weeks (see section 6.1, Table 5). Diaries should be faxed to **832-825-1198** and a copy should be kept in the research chart.

5.2.3 Measurement of Trough VPA Concentrations

Because sampling true trough concentrations prior to the early morning dose may be logistically difficult, we recommend that VPA trough concentrations should be sampled 30 minutes to an hour prior to the afternoon dose.

Once a patient has reached the targeted trough concentration 85-115 mcg/ml, VPA trough concentrations will be measured weekly during radiation therapy, and then monthly or as clinically indicated thereafter.

5.3 Bevacizumab Administration and Monitoring Guidelines

5.3.1 Drug Administration

All patients will receive bevacizumab (10 mg/kg iv) every two weeks for a maximal duration of therapy of 24 months (including XRT). The initial infusion time will be over 90 minutes. If there is no reaction following the first dose, the second dose will be given over 60 minutes, and if tolerated, all subsequent doses will be given over 30 minutes. Immediately after completion of the infusion, flush the IV infusion line with 30 mL of 0.9% sodium chloride to run at the same rate as the bevacizumab infusion.

5.3.2 Monitoring Guidelines

a. Use anaphylactic precautions
b. Monitor vital signs prior to the infusion, every 15 minutes for the first 30 minutes of the infusion, and then every 30 minutes until completed.
c. See Table 3 for actions to be taken in the event of an infusional reaction.

5.3.3 Premedication

Pre-medication is not required for the first dose of bevacizumab. If a subject experiences an infusion–associated adverse event, acetaminophen, steroids, diphenhydramine, or other medications may be given for symptom control and as premedication for the next 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 minutes as long as the subject continues to be premedicated. If a subject experiences an infusion-associated adverse event with the 60-minute infusion despite pre-medication, all subsequent doses should be given over 90 minutes. Similarly, if a subject experiences an infusion-associated adverse event with the 30-minute infusion, all subsequent doses should be given over 60 minutes.

5.4 Valproic Acid Dose Modifications

Toxicities will be graded according to the NCI Common Terminology Criteria for Adverse Events v3.0 (CTCAE) http://ctep.cancer.gov/reporting/ctc.html. The following dose modifications are for adverse events that are possibly, probably or definitely related to VPA.

5.4.1 Criteria for Permanent Discontinuation of VPA

a. Radiographic evidence of new or progressive CNS hemorrhage, except for punctate size lesions without clinical symptoms
b. ≥ Grade 2 CNS hemorrhage (excluding stable, pre-existing hemorrhage)
c. ≥ Grade 3 non-CNS hemorrhage
d. Evidence of bone marrow aplasia or myelodysplastic syndrome on a bone marrow examination
e. Need for anti-coagulation therapy
f. Female patients becoming pregnant while on study
g. Radiographic evidence of pancreatitis OR ≥ grade 2 elevation in amylase and/or lipase, accompanied by abdominal pain, jaundice, vomiting, anorexia, or other symptoms possibly attributable to pancreatitis, in the absence of other apparent etiologies, such as, but not limited to, trauma or gall stones
h. ≥ grade 3 encephalopathy, with or without hyperammonemia

5.4.2 Criteria for Discontinuation of VPA During XRT

a. Interruption of planned radiation, other than mechanical or logistical reasons, for greater than 5 consecutive days or for greater than 10 days total.
b. Any VPA-related adverse event, regardless of grade, that results in the need for permanent cessation of VPA therapy (see Section 5.4.1).
c. Occurrence of DLT despite maintaining VPA trough concentration < 85 mcg/ml (see section 5.4.5 below)

5.4.3 Dose Modifications for other Non-Hematological Toxicity During XRT

5.4.3.1 VPA should be withheld if the patient experiences the following:

a. Any grade 4 non-hematological toxicity
b. Any grade 3 non-hematological toxicity except:

- Grade 3 nausea and vomiting of fewer than 5 days in duration
- Grade 3 transaminase elevation that returns to levels meeting initial eligibility criteria within 7 days of VPA interruption and does not recur upon re-challenge with VPA
- Grade 3 fever or infection of fewer than 5 days in duration
- Grade 3 hypophosphatemia, hypokalemia, hypocalcemia or hypomagnesemia responsive to oral supplementation

c. Any grade 2 non-hematological toxicity that persists for > 14 days and is considered medically significant or sufficiently intolerable by patients such that it requires treatment interruption
d. Any VPA-related adverse event requiring interruption of drug for > 14 days or which recurs upon drug re-challenge.

5.4.3.2 If the toxicity resolves to meet study eligibility parameters within 14 days of discontinuation, the patient may resume treatment at a reduced dose as outlined in Section 5.4.5.

5.4.4 Dose Modifications for Hematological Toxicity During XRT

5.4.4.1 VPA should be withheld, and CBCs should be monitored at least twice weekly if the patient experiences the following:

a. Grade 4 neutropenia (< 500/mm^3)
b. Grade 3 thrombocytopenia (platelets 25,000 to 50,000/mm^3) on two consecutive determinations at least 7 days apart
c. Grade 4 thrombocytopenia (platelet < 25,000/mm^3)

5.4.4.2 If the toxicity resolves to meet study eligibility parameters within 14 days of discontinuation, the patient may resume treatment at a reduced dose as outlined in Section 5.4.5.

5.4.5 Restarting VPA After Treatment Interruption

a. VPA will be re-started at 5 mg/kg/day lower than the previous dose (for example, if a patient's VPA was at 15 mg/kg/day divided tid at the time of the toxicity, then he/she should re-start at 10 mg/kg/day divided tid).
b. VPA trough concentrations should then be monitored every 3-5 days (if day 3 falls on a weekend), and dose adjustments in 5 mg/kg will be made to **maintain a trough concentration between 50-85 mcg/ml in the event of a dose reduction.**
c. If a patient experiences another toxicity requiring that VPA be interrupted or held, despite maintaining the trough VPA concentration below 85 mcg/ml, then he/she will complete the remainder of radiation therapy without VPA.

5.4.6 VPA Dose Modification During Post-XRT Period and Maintenance Therapy

a. For patients that tolerated VPA during XRT, they will continue VPA during the post-radiation break and maintenance therapy to maintain the trough concentration previously tolerated. Guidelines outlined in section 5.4.1

through 5.4.5 will be implemented for any VPA-related toxicities during the post-XRT period and during maintenance therapy.

b. For patients that experience a VPA-related toxicity during maintenance therapy, bevacizumab will be administered on schedule, provided that there is no other contraindication for bevacizumab (see section 5.5 and Table 3).
c. Patients who experience VPA-related toxicities during maintenance therapy despite maintaining trough concentration below 85 mcg/ml will complete maintenance therapy without re-starting VPA.
d. If VPA is discontinued during XRT (except for any reason outlined in Section 5.4.1), **VPA should be re-started with the first day of maintenance treatment**. The starting dose should be 10 mg/kg/day divided tid (or 5 mg/kg/day if patient experienced toxicities previously with 10 mg/kg/day). Dose escalations in increments of 5 mg/kg/day can then be made as outlined in Section 5.2.2 and 5.2.3 to **maintain trough VPA concentration of 50-85 mcg/ml**. Dose modification will be conducted as outlined in 5.4.1 through 5.4.5.
e. For VPA interruption to accommodate an elective surgical procedure, see section 5.7.3b.

5.5 Bevacizumab Dose Modifications

Toxicities will be graded according to the NCI Common Terminology Criteria for Adverse Events v3.0 (CTCAE) http://ctep.cancer.gov/reporting/ctc.html. The following dose modifications are for adverse events that are possibly, probably or definitely related to bevacizumab.

5.5.1 There will be no dose reductions for toxicity related to bevacizumab. See Table 3 for actions to be taken for bevacizumab related adverse events. If bevacizumab is held and a patient cannot resume treatment before the stipulated time for each toxicity, then this patient will discontinue bevacizumab.

5.5.2 See Appendix V for other toxicities that may possibly, probably or definitely attributable to bevacizumab. If a toxicity can be clearly attributed to bevacizumab, VPA will be continued while bevacizumab is being held. In the event that a toxicity may be attributable to either VPA or bevacizumab and requires treatment modification, then **BOTH** VPA and bevacizumab treatment will be modified accordingly.

5.5.3 For bevacizumab interruption to accommodate an elective surgery, see section 5.7.3a.

TABLE 3: Recommended Action for Bevacizumab Related Adverse Events		
Event	**CTCAE.v3.0 Grade**	**Action to be Taken**
Allergic reactions, **or** **Acute infusional reactions/cytokine release syndrome**	Grade 1-3	If infusion-related or allergic reactions occur, premeds should be given with the next dose, and infusion time may not be reduced for the subsequent infusion. **For patients with Grade 3 reactions**, Bevacizumab infusion should be stopped and not restarted on the same day. At the physicians' discretion, bevacizumab may be permanently discontinued or re-instituted with premeds and at a rate of 90±15 min. **If bevacizumab is re-instituted, the patient should be closely monitored for a duration comparable to or longer than the duration of the previous reactions.**
	Grade 4	Discontinue bevacizumab
Arterial Thrombosis ▪ Cardiac ischemia/infraction ▪ CNS ischemia (TIA, CVA) ▪ Any peripheral or visceral arterial ischemia/thrombosis	Any	Discontinue bevacizumab
Venous Thrombosis	Any	Discontinue bevacizumab
Hypertension	[Treat with anti-hypertensive medication as needed. The goal of BP control should be consistent with general medical practice]	
	Controlled BP	Continue bevacizumab
	Persistent or symptomatic HTN	Hold bevacizumab. If treatment is delayed for > 4 weeks due to uncontrolled hypertension, discontinue bevacizumab.
	Grade 4	Discontinue bevacizumab
Proteinuria	Proteinuria should be monitored by urine analysis for urine protein creatinine (UPC) ratio prior to every other dose of bevacizumab. If UPC ratio is >1.0, obtain 24-hour urine collection for protein estimation	
	Urine protein < 3.5 gm/24 hrs	Continue bevacizumab.
	UPC protein ≥ 3.5gm/24 hrs	Hold bevacizumab until urine protein is < 3.5 gms / 24 hours. If therapy is held for > 8 weeks due to proteinuria, discontinue bevacizumab.
	Grade 4 or nephrotic syndrome	Discontinue bevacizumab.

TABLE 3: Recommended Action for Bevacizumab Related Adverse Events		
Event	**CTCAE.v3.0 Grade**	**Action to be Taken**
CNS hemorrhage (except punctuate lesions)	Any Grade	Discontinue bevacizumab.
Hemorrhage	Grade 3	• Hold bevacizumab until ALL of the following criteria are met: ▪ the bleeding has resolved and Hgb is stable. ▪ there is no bleeding diathesis that would increase the risk of therapy. ▪ there is no anatomic or pathologic condition that could increase the risk of hemorrhage recurrence. • Patients who experience recurrence of grade 3 hemorrhage should discontinue study therapy. •
	Grade 4	Discontinue bevacizumab.
Wound dehiscence requiring medical or surgical intervention		Discontinue bevacizumab.
Reversible Posterior Leukoencephalopathy syndrome (RPLS)		• **BV should be held in patients with symptoms/signs suggestive of RPLS, pending work-up and management, including control of blood pressure. BV should be discontinued upon diagnosis of RPLS.**
GI perforation, GI leak or fistula		Discontinue bevacizumab.
Metaphyseal dysplasia (on plain X-ray or MRI)		Discontinue bevacizumab
Other clinically significant AEs attributable to bevacizumab (except controlled nausea/vomiting).	Grade 3	• Hold bevacizumab until symptoms resolve to ≤ grade 2. • If treatment delay is > 4 weeks due to toxicity, discontinue bevacizumab.
	Grade 4	• Discontinue bevacizumab. • **Upon consultation with the study chair**, resumption of bevacizumab may be considered if a patient is benefiting from therapy, and the G4 toxicity is transient, has recovered to ≤ grade 2 and unlikely to recur with retreatment.

5.6 Discontinuation of Either VPA or Bevacizumab During Maintenance Treatment

If either VPA or bevacizumab must be discontinued during maintenance treatment as defined in section 5.4 or 5.5, patients/parents and treating physicians have the option of keeping patients on study and continue receiving either agent alone. Patients/parents may also choose to come off treatment and seek other therapeutic options.

5.7 Supportive Care

5.7.1 Infusional reactions

Routine premedication is not required for the first dose of bevacizumab. If infusional reactions occur, then the infusion should be stopped and acetaminophen, diphenhydramine, steroids, or other medications may be given for symptom control and for premedication as needed (see section 3.2.5 for adjustment of infusion length). Anaphylactic precautions should be observed during bevacizumab administration.

5.7.2 Hypertension

Patients will have their blood pressure monitored every 2 weeks prior to each infusion of bevacizumab. Anti-hypertensive therapy should be initiated and increased as needed per routine practice to maintain SBP and DBP < 95% for age (see Appendix III).

5.7.3 Surgery and wound healing:

a. Bevacizumab-related concerns:

If patients on treatment with bevacizumab require elective major surgery (see Table 4), it is recommended that bevacizumab be held for 4-8 weeks prior to the surgical procedure. Patients undergoing a major surgical procedure should not 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 bevacizumab not to be restarted until 8 weeks after surgery). For elective intermediate surgical procedures (see definition below), bevacizumab should be discontinued at least 2 weeks prior to surgery, and for elective minor procedure, bevacizumab should be discontinued at least 7 days prior to surgery.

Table 4: Examples of Major, Intermediate, or Minor Surgical Procedures

Major	Intermediate	Minor Procedures
Major craniotomy for tumor resection	Central line placement	Incision and drainage of superficial skin abscess
Organ resection	Paracentesis	Punch biopsy of skin lesions
Bowel wall anastamosis	Thoracocentesis	Superficial skin suturing
Arteriovenous grafts		Bone marrow aspirate or biopsy
Exploratory laparotomy		Fine needle aspiration
Thoracotomy		PICC line placement

b. Valproic acid-related concerns:
Valproic acid can adversely affect platelet function and coagulation. For all elective surgical procedures, VPA should be withheld for a minimum of seven days, and pre-operative PT, PTT, platelet aggregation study, fibrinogen, and a von Willebrand panel should be performed. Prophylactic platelet transfusions should be considered for patients with a platelet count < 100,000/ mm^3. Patients with any abnormal coagulation parameters should receive appropriate supportive care with blood or factor support. VPA should not be resumed until patient's risk for post-operative bleeding is deemed minimal by the surgeons, and VPA should be started at the same dose as patient was receiving prior to surgery. Patients undergoing emergent surgical procedures should be supported as clinically indicated.

5.7.4 Proteinuria: Proteinuria will be monitored by urine protein:creatinine (UPC) ratio (see Appendix II) at least every 4 weeks.

5.7.5 Central venous catheters: Treatment via central venous catheters is recommended but not required. Placement of central venous catheters should be performed at least 2 weeks prior to initiating bevacizumab. Placement of a PICC line should be performed at least 48 hours before starting bevacizumab.

5.7.6 Concomitant medications:
a. Concomitant use of other anti-convulsants is strongly discouraged.
b. The routine use of growth factors (GCSF, GM-CSF, and erythropoietin) is not permitted, except for febrile neutropenia and/or sepsis.
c. Appropriate antibiotics, blood products, antiemetics, fluids, electrolytes, and general supportive care are to be used as deemed clinically necessary by the treating physicians.
d. Concomitant medications that may impact platelet function (e.g., ibuprofen or aspirin) or decrease platelet number should be avoided.
e. See section 3.1.8 for VPA interactions with other medications.

5.7.7 Elevated MCV: In the event of a persistent elevation of MCV > 2 SD of age-adjusted mean for two consecutive determinations at least 2 weeks apart **and** persistent ≥ grade 3 cytopenias of at least two cell lines for at least 2 weeks, VPA should be withheld, and a bone marrow examination should be performed to rule out bone marrow aplasia or myelodysplastic syndrome.

5.7.8 Hepatitis/pancreatitis: In the event of unexplained abdominal pain, jaundice, vomiting, anorexia, weight loss, or other symptoms possibly attributable to hepatitis and/or pancreatitis, amylase/lipase, ALT, bilirubin, fibrinogen, and an abdominal ultrasound should be performed immediately, and VPA should be withheld pending the results of these tests.

Patients who experience radiographic documentation of pancreatitis OR ≥ grade 2 elevation in amylase/lipase accompanied by abdominal pain, jaundice,

vomiting, anorexia, or other symptoms possibly attributable to pancreatitis, in the absence of other apparent etiologies, such as, but not limited to, trauma or gall stones, will discontinue VPA for the remainder of protocol therapy.

5.7.9 Hyperammonemia, encephalopathy, and carnitine supplementation: Hyperammonemia has been reported in association with VPA despite normal liver function tests. In patients who develop unexplained lethargy, vomiting, and/or changes in mental status, hyperammonemic encephalopathy should be considered, and an ammonia level should be checked immediately. In patients with ≥ grade 3 encephalopathy, with or without hyperammonemia, VPA should be discontinued for the remainder of protocol therapy, and evaluations for urea cycle disorders should be considered, if clinically indicated. In patients with hyperammonemia encephalopathy, intravenous L-carnitine should be considered with other supportive measures (109, 110).

Individual investigators may elect to supplement L-carnitine, at 50-100 mg/kg/day, divided bid or tid, not to exceed 3 gram/day. Carnitine supplementation has been shown to ameliorate the CNS symptoms in those children with carnitine depletion as a result of VPA administration.

6.0 EVALUATION AND MEASUREMENT TO BE OBTAINED

6.1 Required Observation During Protocol Therapy

Laboratory tests to determine eligibility for protocol therapy must be obtained within 7 days of study registration/entry. MRI of brain +/- spine must be obtained within 2 weeks prior to study registration/entry and **must include gradient echo imaging (GRE) sequences for identifying CNS/intra-tumoral hemorrhage**. Histologic verification of tumors is required for all patients, except for those with a typical intrinsic brainstem glioma (see section 4.1).

TABLE 5: Clinical, Laboratory, and Radiographic Assessments Required for Protocol Therapy

	Pre-XRT and XRT Phase		Post -XRT		Maintenance Treatment				
	Study Entry	**Q Wk**	**Q 2Wks**	**Week 10**	**Pre -Rx**	**Q 2Wks**	**Q 4Wks**	**Q 12 Wks**	**Q 24 Wks**
PHYSICAL EXAM									
History & Physical, including neuro exam	X	X	X		X		X[1]		
Vital Signs,	X	X	X		X		X[1]		
Performance Status	X	X	X		X		X		
Height/Weight/BSA	X	X	X		X		X		
Blood Pressure	X	X	X		X	X			
LABORATORY									
CBC, diff, platelets	X	X[2]	X[2]		X[2]		X[2]		
Lytes. BUN/Cr, Albumin	X				X		X		
ALT, AST, bilirubin	X	X[2]	X[2]		X		X[2]		
Amylase & Lipase	X				X				
Urine protein/Cr[3]	X[3]				X[3]		X[3]		
PT, PTT & INR	X				X				
Pregnancy test[4]	X[4]				X[4]				
CSF cytology	X[5]			X[5]				X[5]	
Valproic acid levels[6]	See Guidelines in Sections 5.2.3								
Correlative Biology	X[7]								
Imaging Studies									
MRI of brain	X			X[8]			X[10]	X[9]	
MRI of spine	X[11]			X[11]				X[11]	
X-ray of right knee[12]					X[12]				X[12]
Patient Diary[13]		X[13]	X[13]		X[13]	X[13]	X[13]		

[1] For patients with newly identified punctate hemorrhage on MRI, physical exam, neurological assessment, and performance status should be evaluated every 2 weeks at least twice to closely monitor for signs/symptoms of worsening hemorrhage.

[2] Patients with grade 4 neutropenia, anemia, and/or thrombocytopenia should have CBC checked twice weekly until the cytopenia(s) improves to grade 3 or better. Patients who require a subsequent dose escalation to maintain targeted trough concentration should also have trough VPA, CBC, and liver functions measured every 3-5 days until target is reached.

[3] See Appendix II for Urine/Protein Creatinine ratio

[4] For female patients of child-bearing potential

[5] CSF cytology at the time of diagnosis and subsequently during protocol therapy should be performed as clinically indicated but is not required for study entry.

[6] See Section 5.2.3 for monitoring valproic acid concentrations.

[7] See section 7.0 for requirement and timing for correlative biology studies.

[8] For patients with possible radiographic progression on week 10 MRI, the next MRI should be performed at week 18 (see section 8.3). If week 18 MRI shows stable disease or better compared to week 10 MRI, then patients will continue MRI every 12 weeks.

[9] During maintenance therapy, MRI of brain, including GRE images, should be performed every 12 weeks until completion of protocol therapy.

[10] For patients with newly identified punctate hemorrhage on MRI, the next MRI, including GRE images, should be repeated every 4 weeks at least twice to monitor possible progression of hemorrhage. In the absence of radiographic progression of hemorrhage, the frequency of MRI studies can then return to every 12 weeks.

[11] For patients with high-grade gliomas, an MRI of spine should be performed at the time of study entry, but subsequent MRI of spine should only be repeated as clinically indicated.

[12] Pre-treatment tibial X-ray (AP and lateral views) of the right knee should be obtained in all patients younger than 16 years of age prior to starting maintenance therapy and repeated every 24 weeks while receiving bevacizumab. If abnormalities of the metaphyseal plates are detected on routine X-rays following initiation of treatment, an MRI scan of both knees should be performed.

[13] Medication diaries and treatment roadmaps should be checked and faxed weekly during radiation therapy. After completion of XRT, medication diaries and treatment roadmaps should be checked every 2 weeks and faxed every 4 weeks. Diaries should be faxed to 832-825-1198. Maintain copies of the patient diary in the research chart.

6.2 Required Observations After Completion of Protocol Therapy

After completion of protocol therapy, patients should have the following assessments and laboratory tests every 6 months for a minimum of 5 years, or until off study (Section 10.5): history and physical examination, vital signs, including blood pressure, CBC with differential, urine protein:creatinine ratio, and MRI to assess disease status. Obtain all other tests as required for good clinical care. Complete Appendix VII: Protocol Roadmap for Follow-up and Fax to 832-825-1198 every six months.

7.0 BIOLOGIC CORRELATIVE STUDIES

7.1 Frozen Tissue Submission

7.1.1 Proteins related to histone deacetylation (histone H3 and H4 acetylation, HDAC 1 and 2), NHEJ (Ku70/Ku80, DNA-PK) and HRR (Rad51, BRCA1/2) pathway, tumor angiogenesis (VEGF, VEGF receptor 2), and tumor hypoxia (CD31, CA9, hypoxia-inducible factor 2α) will be quantified by Western analysis if sufficient frozen tumors resected at diagnosis and/or at later time points are available.

7.1.2 MGMT and PARP activity will be assessed by enzymatic assays in frozen tumors, if available.

7.1.3 **Submission of frozen tumor specimens is strongly encouraged. A minimum of 250 mg (wet weight prior to being frozen in liquid nitrogen; approximately 1.5 x 1.5 x 1.5 cm of tumor sample) of tumor specimen is preferred. Please coordinate with neurosurgery and pathology in advance to ensure timely collection and freezing of tumors.** In patients whose tumors are only biopsied, submission of as much frozen tumor specimen as possible is strongly encouraged, and Formalin-fixed, paraffin-embedded (FFPE) tumor blocks should also be submitted.

7.1.4 Frozen tumors should be shipped on dry ice via over-night express delivery on Monday through Thursday. If a tumor is resected on Friday or over the weekend, the frozen specimen should be kept at -80^0C over the weekend and shipped on Monday. FFPE tumor blocks and unstained tumor section slides should be shipped at room temperature. Specimen Transmittal Form (Appendix VI) must accompany all shipments. All specimens should be shipped to the following address:

Jack Su, MD
Attention: Elizabeth Hinojosa
Feigin Center, Baylor College of Medicine
1102 Bates Street, Rm 1030
Houston, Texas 77030
(832) 824-4688 or (832) 822-4306

7.2 Slides for Immunohistochemistry Studies

7.2.1 In patients with insufficient frozen tumor obtained for analysis of molecular markers outlined in 7.1.1, similar analysis will be performed by immunohistochemistry (IHC) in FFPE tumor blocks. **Ten to twelve unstained**

PLUS glass slides, containing 5-micron thick tumor sections from FFPE tumor blocks will be requested for performing studies outlined in 7.1.1.

7.2.2 Specimen Transmittal Form (Appendix VI) must accompany all shipments. Slides should be shipped to Dr. Jack Su at the address provided in Section 7.1.4.

7.3. Blood Samples Submission

7.3.1 NHEJ activity, HDAC2 level, and histone acetylation in PBMCs: PBMCs will be isolated using Lymphoprep solution (Axis-Shield, Oslo, Norway), and protein lysates will be prepared by standard techniques. NHEJ activity will be analyzed via a published assay (119). HDAC2 level will be quantified using Western analysis. Histone acetylation will be quantified using either Western analysis or a PathScan® Acetylated Histone H3 and H4 Sandwich ELISA Kit (Cell Signaling, Danvers, MA).

7.3.2. Sampling Schedule (See Appendix VI)
Blood samples should be collected prior to starting VPA and radiation, and then after completing two weeks of VPA and radiation (any weekday, except Friday, during 3rd week of radiation).

7.3.3. Sample Collection and Handling Instructions
Whole blood samples (a minimum of 5-10 ml for children < 12 kg, and 10-20 ml for children ≥ 12 kg) should be collected in either **heparinized (green tops, either sodium or lithium heparin is acceptable) or ACDA (yellow top) tubes.** ACDA tubes are preferred if available. **DO NOT collect blood samples on a Friday.**

7.3.4. Sample Processing
No processing is required. Keep the whole blood at room temperature until the time of shipping.

7.3.5. Sample Labeling
Each tube must be labeled with the patient's study ID number, and the date and time the sample was drawn. Data should be recorded on the Correlative Study Form (Appendix VI), which must accompany the sample(s).

7.3.6. Sample Shipping Instructions
Each sample should be shipped immediately on the day of collection. Please follow packaging instruction on the Specimen Transmittal Form (Appendix VI) Samples should be shipped by priority overnight via express carrier (**Monday through Thursday only**). Between April and October, please ship with an ice pack placed in the Styrofoam box (ice pack should not be placed directly next to the samples).

Jack Su, MD
Attention: Elizabeth Hinojosa
Feigin Center, Baylor College of Medicine
1102 Bates Street, Rm 1030
Houston, Texas 77030

(832) 824-4688 or (832) 822-4306

Prior to shipping, please contact Dr. Jack Su (832-822-4306 or jmsu@txccc.org), Dr. Li (832-824-4580 or xxli@txccc.org), or Elizabeth Hinojosa (832-824-4688) for notification of sample shipment.

8.0 EVALUATION CRITERIA

8.1 Response Criteria for Patients with Measurable CNS Tumors

8.1.1 Measurable Disease

The presence of at least one lesion that can be accurately measured in at least one dimension with the longest diameter at least 10 mm. The investigator will identify up to 10 measurable lesions to be followed for response.

8.1.2 Complete Response (CR)

No evidence of disease at the primary tumor site. Patient is not on any steroids with stable or improving neurological examination.

8.1.3 Partial Response (PR)

A greater than 50% reduction in the product of the greatest tumor diameter and its perpendicular diameter on MRI scan, on a stable or decreasing dose of steroids with a stable or improving neurologic examination.

8.1.4 Minor Response (MR)

A greater than 25% but less than 50% reduction in the product of the greatest tumor diameter and its perpendicular diameter on MRI scan, on a stable or decreasing dose of steroids with a stable or improving neurologic examination.

8.1.5 Stable Disease (SD)

A less than 25% reduction in the product of the greatest tumor diameter and its perpendicular diameter on MRI scan, on a stable or decreasing dose of steroids with a stable or improving neurologic examination.

8.1.6 Progressive Disease (PD)

Progressive Disease (PD) will be defined as a more than 25% increase in tumor size radiographically or the emergence of new lesions or CSF positivity.

8.2 Response Criteria for Patients with Evaluable CNS Tumors

8.2.1 Evaluable Disease

The presence of at least one lesion that cannot be accurately measured in at least one dimension (< 10 mm). Such lesions may include but are not limited to leptomeningeal tumor deposits and CSF positivity for tumor cells.

8.2.2 Complete Response

Disappearance of all evaluable disease.

8.2.3 Partial response

Partial responses cannot be determined in patients with evaluable disease

8.2.4 Stable Disease

Any response that does not meet the criteria for complete response or progressive disease.

8.2.5 Progressive Disease

The appearance of one or more new lesions or evidence of laboratory, clinical, or radiographic progression.

8.3 Best Response

Two objective determinations of disease status by MRI, obtained on two consecutive determinations, separated by at least a four week time period, are required to determine the patient's overall best response (see Table 6). Two objective status determinations of CR before progression are required for best response of CR. Two determinations of PR or better before progression, but not qualifying for a CR, are required for a best response of PR. Two determinations of stable/no response or better before progression, but not qualifying as CR or PR, are required for a best response of stable/no response; if the first objective status is unknown, only one such determination is required. Patients with an objective status of progression on or before the second evaluations will have a best response of progressive disease. Best response is unknown if the patient does not qualify for a best response of progressive disease and if all objective statuses after the first determination and before progression are unknown.

Due to radiation related edema and inflammation, patients can show radiographic images suggestive of disease progression within the first two months after radiation, and these MRI findings then improve or resolve over the next 6-8 weeks. Therefore, for patients whose MRI images at week 10 suggest possible tumor progression, they should remain on study, start maintenance therapy, and repeat MRI around week 18. Patients with stable disease or better at week 18 (compared to week 10) will continue protocol therapy, and patients with progressive disease will come off protocol treatment.

Table 6. Sequences of objective statuses with corresponding best response.

1st Status	2nd Status	3rd Status	Best Response
Progression[1]			Progressive disease
Stable, PR, CR	Progression		Progressive disease
Unk	Progression		Progressive disease
Stable	Stable	Progression	Stable
Stable, Unk	PR, CR	Progression	Stable
Stable, Unk	Unknown	Progression	Unknown
PR	PR	Progression	PR
PR	CR	Progression	PR
PR, CR	Unknown	Progression	Unknown
CR	CR	Progression	CR
Unknown	Stable	Progression	Stable

[1] Except if week 10 MRI shows progressive disease, then the repeat MRI obtained around week 18 will be compared to week 10 MRI for the first response assessment.

9.0 RADIATION THERAPY GUIDELINES

Radiation therapy must begin within 30 days of surgery or radiographic diagnosis, whichever is the later date. Date of radiographic diagnosis or definitive surgical procedure is considered day 1, and radiation treatment must start no later than day 31. Primary brain malignant gliomas will receive a total dose of between 54.0 and 59.4 Gy in 30-33 fractions over 6-7 weeks. The total dose will be 54.0 Gy for completely resected tumors and brainstem gliomas. The total dose will be 59.4 Gy if the tumor is located in the brain but not the brainstem, and the tumor was incompletely resected. Primary spinal cord malignant gliomas will receive a total dose of between 50.4-54 Gy in 28-30 fractions over 5 - 6 weeks.

9.1 Equipment

Modality: X-rays with nominal energy of 4 MV or greater or proton beam.

9.2 Target Volumes

RT volumes for this study will be determined by the collective information that delineates the extent of disease both prior to and following surgical resection or biopsy. The required imaging studies are a contrast-enhanced pre-operative MRI scan, a contrast-enhanced post-operative MRI scan (unless only a biopsy was performed), and a radiation treatment planning CT scan with the patient in the treatment position. Use of fusion image registration software is encouraged, if available.

9.2.1 Gross Tumor Volumes (GTV)

The GTV-1 will include all the tissues initially involved with disease and the entire residual tumor defined by the pre-operative and post-operative MRI scans. The GTV-1 will include both enhancing and non-enhancing areas of the tumor. The GTV-1 will take into account any changes in brain anatomy that have occurred as a result of tumor resection and/or CSF shunt placement. T-1, T-2, and FLAIR sequences of the MRI scans should all be reviewed, and the sequence that best defines the extent of initial disease should be used to determine the GTV-1. The GTV-2 will include only the residual tumor seen on the post-operative MRI scan. The GTV-2 will include both enhancing and non-enhancing areas of the residual tumor. T-1, T-2, and FLAIR sequences of the post-operative MRI scan should all be reviewed, and the sequence that best defines the extent of residual disease should be used to determine the GTV-2. If only a small biopsy has been performed, the GTV-2 may be identical to the GTV-1. If the tumor has been completely resected, there will not be a GTV-2 and the radiation therapy course will end with completion of the initial radiation fields.

9.2.2 Clinical Target Volumes (CTV)

The CTV includes the GTV with an added margin that is intended to treat subclinical microscopic disease and is anatomically confined. This means that margins of the CTV may be manually moved inward to the inner table of the bony calvarium or (for spinal cord tumors) the spinal canal. The CTV-1 will include the GTV-1 plus a 2 cm margin in all dimensions. For primary spinal cord tumors, the CTV-1 will be expanded in the cranial and caudal directions to include 2 vertebral bodies above and 2 vertebral bodies below the GTV-1. The CTV-2 will include the GTV-2 plus a 1 cm margin in all dimensions. For primary spinal cord tumors, the CTV-2 will be expanded in the cranial and caudal directions to include 1 vertebral body above and 1vertebral body below the GTV-2. If there is not a GTV-2, there will not be a CTV-2.

9.2.3 Planning Target Volumes (PTV)

The PTV includes the CTV with an added margin that is intended to account for patient movement and set-up variability. The treating radiation oncologist will select a margin between 3 and 5 mm that reflects the variability appropriate for the individual patient and facility. The PTV-1 will include the CTV-1 plus a 3-5 mm margin in all dimensions. The PTV may extend beyond bone margins, but shall not extend beyond the skin surface. The PTV-2 will include the CTV-2 plus a 3-5 mm margin in all dimensions. The PTV may extend beyond bone margins, but shall not extend beyond the skin surface. If there is not a CTV-2, there will not be a PTV-2.

9.3 Target Dose

9.3.1 Prescription Point

The prescription point is at or near the isocenter. If IMRT is used, dose may be prescribed to an isodose surface that encompasses the PTV provided that the dose uniformity requirements in Section 9.3.5 are satisfied.

9.3.2 Dose Definition

Dose is specified in Gy to muscle.

9.3.3 Tissue Heterogeneity

Calculations that take into account tissue heterogeneity are required for CT-based planning techniques.

9.3.4 Prescription Point and Fractionation

9.3.4.1 Planning Target Volume 1 (PTV-1)

For brain tumors, the total dose to the PTV-1 prescription point will be 54 Gy given in 30 fractions of 1.8 Gy each. For spinal cord tumors, the total dose to the PTV-1 prescription point will be 45 Gy given in 25 fractions of 1.8 Gy each. The patient will be treated with one fraction per day. All fields will be treated each day. The total dose to the prescription point will be 54 Gy given in 30 fractions. The patient will be treated with one fraction per day with all fields treated per day. 1.80 Gy will be delivered to the isocenter.

9.3.4.2 Planning Target Volume 2 (PTV-2)

This boost is delivered only to those patients with gross residual disease other than brainstem tumors. For brain tumors with gross residual disease, the total boost dose to the PTV-2 prescription point will be 5.4 Gy given in 3 fractions. The cumulative dose to the prescription point will be 59.4 Gy. The patient will be treated with one fraction per day. All fields will be treated each day. For spinal cord tumors with gross residual disease, the total boost dose to the PTV-2 prescription point will be between 5.4 Gy given in 3 fractions and 9 Gy given in 5 fractions. The treating radiation oncologist will prescribe between 3 and 5 fractions based on his/her practice. The cumulative dose to the prescription point will be between 50.4 and 54 Gy.

9.3.5 Dose Uniformity
For standard planning techniques, the dose variation in the PTV shall be within +7% and -5% of the prescribed dose. For conformal planning techniques (3D conformal and IMRT), the entire PTV shall be encompassed within the 95% isodose surface and no more than 10% of the PTV should receive more than 110% of the prescription dose, as evaluated by dose volume histogram.

9.3.6 Treatment Interruptions
No treatment breaks in the radiation therapy component of this combined modality treatment are anticipated. Skin reactions should be treated supportively. Low blood counts are generally related to systemic therapy and are not caused or worsened by local field brain RT.

9.4 Treatment Technique

9.4.1 Treatment Planning
Two-dimensional or conformal (three dimensional) planning may be used in this study.

9.4.2 Patient Position and Immobilization
Reproducible setups are critical and the use of immobilization devices such as thermoplast mask, biteblock, etc. should be used for all pediatric patients and must be used for patients being treated with IMRT.

9.4.3 Field Shaping
Field shaping can be done with blocks or multi-leaf collimation.

9.5 Normal Tissue Sparing

9.5.1 Lenses of the Eyes
The lenses of the eyes must be excluded from the primary beam by the use of shielding blocks that are at least 5 HVL thick.

9.5.2 Optic Nerve and Chiasm
Whenever possible without shielding gross tumor, the dose to the optic nerves and chiasm should not exceed 54.0 Gy and the dose to the retinas of the eyes should not exceed 45.0 Gy, including the dose from the boost.

9.5.3 Spinal Cord
The dose to the spinal cord within the PTV shall not exceed 54 Gy. The dose to the spinal cord more than 5 mm outside the PTV should not exceed 46 Gy.

10.0 STATISTICAL CONSIDERATION

10.1 Sample Size and Study Duration

There will be two major subgroups of patients:

a. Patients with high-grade gliomas (GBM, anaplastic astrocytoma)
b. Patients with other gliomas (gliosarcoma, gliomatosis cerebri, or diffuse intrinsic brainstem gliomas)

EFS and OS will be analyzed separately for each group.

For high-grade gliomas, 30 patients will be enrolled over 3 years, with a minimum follow-up of 24 months. Using a one-year EFS of 36% for children with GBM in ACNS0126, this sample size will achieve 90% power (using a one-sided $\alpha = 0.05$) in detecting a 20% improvement in one-year EFS (from 36% to 56%) in comparison. If only 21 patients are enrolled, the proposed study will achieve 80% power in detecting a 20% improvement in one-year EFS.

For other gliomas, the 1-year EFS of 17% for children with newly diagnosed brainstem gliomas from CCG-9941 (10) will be used for comparison. If 26 patients from this group are enrolled, this study will achieve 90% power (using a one-sided $\alpha = 0.05$) in detecting a 20% improvement in one-year EFS (from 17% to 37%). If only 19 patients are enrolled, the proposed study will achieve 80% power in detecting a 20% improvement in one-year EFS. These calculations were performed for one-arm survival studies under the assumption of exponentially distributed endpoints.

Based on the number of children newly diagnosed with high-grade gliomas in Texas in 2006, we anticipate an accrual of 1-2 patients with high-grade gliomas per month.

10.2 Study Design and Data Analysis

Toxicities will be described separately for the radiation phase (VPA and radiation) and the maintenance phase (VPA and bevacizumab) treatment.

For the radiation phase of treatment, if more than one-third of patients enrolled (i.e. greater than two of the first three, three of the first six, four of the first nine patients, etc.) required discontinuation of VPA, as outlined in section 5.4.1 and 5.4.2, then all subsequent patients will receive radiation therapy without valproic acid. Patients who required discontinuation of VPA during XRT and subsequent patients who received XRT without VPA because this combination is considered not tolerable (as described previously) will still receive VPA and bevacizumab during maintenance, provided that there is no contraindication to VPA as described in 5.4.1.

For the maintenance phase of treatment, if more than one-third of patients enrolled experienced toxicities that require DISCONTINUATION OF BOTH VPA and bevacizumab (as defined in section 5.4 and 5.5), subsequent patients will not receive this combination of treatment. Dose modifications due to toxicities that may be attributed to a single drug are addressed in sections 5.4-5.6.

The Kaplan-Meier method will be used to estimate the one-year and two-year EFS and OS for each group with 95% confidence intervals. The one-sample logrank test (120) will be used to test for significant improvement in survival compared to the above-referenced historical trials for each group. Similar analyses will be performed for subgroups of children with completed resected and subtotally resected tumors.

Although tumor response is not the primary endpoint of this trial, for patients with residual tumors after initial surgery or at the start of maintenance therapy, tumor response rate to radiation and VPA or to VPA and bevacizumab will be summarized by calculating

response rates along with exact 95% binomial confidence intervals. Response will be documented by MRI at intervals outlined in Table 6.0 or as clinically indicated. Responses will be defined according to the criteria listed in Section 8.0.

10.3 Measurement of Effect

10.3.1 Response Criteria
See section 8.0 for assessment of best tumor response to treatment.

10.3.2 Event Free Survival (EFS)
Interval of time between study entry and documentation of disease progression (clinical or radiographic), secondary malignancy, death from other causes, or date of last follow-up.

10.3.3 Overall Survival
Interval of time between diagnosis and death due to any cause, or date last follow-up.

10.4 Off Treatment Criteria

10.4.1 Disease progression as outlined in Section 8.0
10.4.2 Unacceptable toxicity as outlined in Section 5.4 and 5.5
10.4.3 Clinical progression manifested by deteriorating mental status, new or worsening neurological findings, or other symptoms/findings felt to be consistent with disease progression by the treating physician
10.4.4 Development of a medical or psychiatric illness, that in the investigators judgment renders the patient incapable of further therapy on this protocol.
10.4.5 The patient, parent or legal guardian refuses further treatment on this protocol.
10.4.6 Completion of all protocol defined treatment.
10.4.7 Pregnancy
10.4.8 Second malignant neoplasm
10.4.9 Need for anti-coagulation therapy
10.4.10 Non-compliance that in the opinion of the investigator does not allow for ongoing participation
10.4.11 Treating physician determines that continued participation is not in the patient's best interest.

Patients who have an on-going bevacizumab-related grade 4 or serious adverse event at the time of discontinuation of protocol therapy will be followed until resolution of the event or until the event is considered irreversible.

10.5 Off Study Criteria

10.5.1 Patient, parent or legal guardian withdraws consent for continued participation including documenting follow-up survival status.
10.5.2 Patient death while on study.
10.5.3 Patient lost to follow-up.

10.6 Correlative Biology Studies

We will use descriptive statistics to explore potential correlations between the proposed molecular parameters in section 7.0 with radiographic response rate, EFS, and OS.

11.0 CENTRAL REVIEW

11.1 Pathology Review

A central review (except for patients with brainstem gliomas who do not undergo tumor biopsy) and a consensus on histopathology meeting study definition of high-grade gliomas will be conducted for all patients who have undergone tumor resection/biopsy. All H&E and immunohistochemical slides, ten unstained slides with 5-micron, FFPE tumor sections, and pathology report from the originating institution's neuropathologist must be submitted by express mail to the following address:

Adekunle M. Adesina, MD, PhD
Department of Pathology, Baylor College of Medicine
One Baylor Plaza, Room 286A
Houston, Texas 77030
832-824-2250

Tumor histology will be reviewed by Drs. Adekunle Adesina (Baylor College of Medicine) and Greg Fuller (MD Anderson Cancer Center).

11.2 Imaging Review

Pre- and post-surgery (except for patients with brainstem gliomas who do not undergo tumor biopsy) MRI images should be submitted on a CD-ROM for a central review by pediatric neuro-radiologists as soon as they are available. All subsequent MRI images should also be submitted after each study to be reviewed for tumor response and assessment of intra-tumoral hemorrhage.

Please submit MRI images on CD to:

Suzanne Wheeler
Texas Children's Cancer Center
1102 Bates, Suite 1590
Houston, Texas 77030

12.0 EVALUATION, RECORDING, REPORTING, AND MONITORING OF ADVERSE EVENTS

Safety assessments will be made using the NCI Common Terminology Criteria for Adverse Events v3.0 (CTCAE) http://ctep.cancer.gov/reporting/ctc.html. Adverse event data collection and reporting are done to ensure the safety of patients enrolled in this study as well as those who will enroll in future trials using similar agents.

12.1 DEFINITIONS

12.1.1 Adverse Events

Adverse Events (AEs) are defined as any untoward medical occurrence in a patient that does not necessarily have a causal relationship with either valproic acid or bevacizumab. An AE for this study can therefore be any unfavorable or unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of either valproic acid and/or bevacizumab, whether or not attributable to these agents used in this investigational study.

AEs that are not described in the protocol, in published medical literature, in the patient's informed consent, or in Investigator Brochure are defined as unexpected AEs.

A pre-existing condition should not be reported as an AE unless the condition worsens or episodes increase in frequency during the adverse event reporting period.

12.1.2 Serious Adverse Events

Serious Adverse Events (SAEs) are defined as any untoward medical occurrence that:

- Results in death
- Is life-threatening
- Requires in-patient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability and/or incapacity
- Is a congenital anomaly/birth defect
- Is any important medical event, based on appropriate medical judgment, that jeopardizes the patient and requires medical and/or surgical intervention to prevent one of the outcomes listed above.

Hospital admission for elective/planned surgeries or administration of protocol therapy should not be reported as SAE2 unless an unpredicted complication results in a prolonged hospitalization.

"Death" and "Disease Progression (PD)" should not be reported as SAEs. Death should be reported as an outcome of an SAE (i.e. the specific cause of death should be reported as the SAE).

12.2 ADVERSE EVENT DESCRIPTION

The AE collection/reporting period will begin with the first day of valproic acid treatment. AEs after study registration but prior to the first day of valproic acid will be captured as ongoing concurrent secondary diagnoses and symptoms present at the start of study. AEs will be graded in accordance with the NCI Common Terminology Criteria for Adverse Events v3.0 (CTCAE) http://ctep.cancer.gov/reporting/ctc.html. If not described in the NCI-CTCAE, AEs will be graded according to their severity using the following criteria: grade 1 (mild), grade 2 (moderate), grade 3 (severe), and grade 4 (life-threatening).

The investigator will assign attribution of an AE to valproic acid, bevacizumab, or BOTH, as one of the following categories:

- Unrelated
- Unlikely
- Possible

- Probable
- Definite

12.3 REPORTING ADVERSE EVENTS

All adverse events, regardless of perceived relationship to study treatment, will be reported and recorded on the appropriate CRFs. New AEs and AEs that are ongoing at the off-treatment visit will be followed for 30 days from the patient's receipt of the last dose of protocol therapy, unless they have resolved earlier. SAEs and drug-related AEs ongoing at the end of study will be followed until resolution.

The AE description will include the nature of the experience, the date of onset, the resolution date, the severity of each sign or symptom reported using the NCI-CTCAE (version 3.0), the seriousness of the event, the potential relationship to study treatment, the course of action taken, and the outcome of the experience.

Any serious or immediately life-threatening adverse experience, including those resulting in death, occurring while the patient is receiving either valproic acid or bevacizumab, or within 30 days of the patient's last dose of protocol therapy, regardless of the treating physician's opinion regarding drug relationship, will be reported by telephone and/or e-mail (within 24 hours of the event) to the Study Chair. A written report must be submitted via fax or e-mail within two working days to the study Chair. Individual institutions are responsible for reporting serious adverse events to their institutional review board (IRB) according to each board's required time frame.

All AE communications and reports should be submitted to:

Jack Su, MD, MS
Office telephone: 832-822-4306
Cell phone: 713-858-8129
Fax: 832-825-1503
E-mail: jmsu@txccc.org

Please "cc" Susan Blaney, MD on all AE communications: sblaney@txccc.org

12.4 MONITORING OF ADVERSE EVENTS

The Dan L. Duncan Cancer Center Pediatric Safety Monitoring Committee (Baylor College of Medicine) will monitor the adverse events for this clinical trial.

13.0 RETENTION OF RECORDS

Retain all documentation of adverse events, records of study drug receipt and dispensation, and all IRB correspondence for at least 2 years after the investigation is completed

References

1. Sposto R, Ertel IJ, Jenkin RD*, et al.* The effectiveness of chemotherapy for treatment of high grade astrocytoma in children: results of a randomized trial. A report from the Childrens Cancer Study Group. J Neurooncol 1989;7(2):165-77.
2. Finlay JL, Boyett JM, Yates AJ*, et al.* Randomized phase III trial in childhood high-grade astrocytoma comparing vincristine, lomustine, and prednisone with the eight-drugs-in-1-day regimen. Childrens Cancer Group. J Clin Oncol 1995;13(1):112-23.
3. Pollack IF, Boyett JM, Yates AJ*, et al.* The influence of central review on outcome associations in childhood malignant gliomas: results from the CCG-945 experience. Neuro Oncol 2003;5(3):197-207.
4. Stupp R, Mason WP, van den Bent MJ*, et al.* Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. N Engl J Med 2005;352(10):987-96.
5. Cohen K, et al. Children's Oncology Group ACNS0126 trial, unpublished data. 2007.
6. Hargrave D, Bartels U, Bouffet E. Diffuse brainstem glioma in children: critical review of clinical trials. Lancet Oncol 2006;7(3):241-8.
7. Packer RJ, Prados M, Phillips P*, et al.* Treatment of children with newly diagnosed brain stem gliomas with intravenous recombinant beta-interferon and hyperfractionated radiation therapy: a childrens cancer group phase I/II study. Cancer 1996;77(10):2150-6.
8. Allen J, Siffert J, Donahue B*, et al.* A phase I/II study of carboplatin combined with hyperfractionated radiotherapy for brainstem gliomas. Cancer 1999;86(6):1064-9.
9. Mandell LR, Kadota R, Freeman C*, et al.* There is no role for hyperfractionated radiotherapy in the management of children with newly diagnosed diffuse intrinsic brainstem tumors: results of a Pediatric Oncology Group phase III trial comparing conventional vs. hyperfractionated radiotherapy. Int J Radiat Oncol Biol Phys 1999;43(5):959-64.
10. Jennings MT, Sposto R, Boyett JM*, et al.* Preradiation chemotherapy in primary high-risk brainstem tumors: phase II study CCG-9941 of the Children's Cancer Group. J Clin Oncol 2002;20(16):3431-7.
11. Broniscer A, Iacono L, Chintagumpala M*, et al.* Role of temozolomide after radiotherapy for newly diagnosed diffuse brainstem glioma in children: results of a multiinstitutional study (SJHG-98). Cancer 2005;103(1):133-9.
12. Jakacki RI, Siffert J, Jamison C, Velasquez L, Allen JC. Dose-intensive, time-compressed procarbazine, CCNU, vincristine (PCV) with peripheral blood stem cell support and concurrent radiation in patients with newly diagnosed high-grade gliomas. J Neurooncol 1999;44(1):77-83.
13. Wolff JE, Westphal S, Molenkamp G*, et al.* Treatment of paediatric pontine glioma with oral trophosphamide and etoposide. Br J Cancer 2002;87(9):945-9.
14. Korones DN, Fisher PG, Kretschmar C*, et al.* Treatment of children with diffuse intrinsic brain stem glioma with radiotherapy, vincristine and oral VP-16: a Children's Oncology Group phase II study. Pediatr Blood Cancer 2008;50(2):227-30.
15. Broniscer A, Leite CC, Lanchote VL, Machado TM, Cristofani LM. Radiation therapy and high-dose tamoxifen in the treatment of patients with diffuse brainstem gliomas: results of a Brazilian cooperative study. Brainstem Glioma Cooperative Group. J Clin Oncol 2000;18(6):1246-53.
16. Greenberg ML, Fisher PG, Freeman C*, et al.* Etoposide, vincristine, and cyclosporin A with standard-dose radiation therapy in newly diagnosed diffuse intrinsic brainstem gliomas: a pediatric oncology group phase I study. Pediatr Blood Cancer 2005;45(5):644-8.
17. Dunkel IJ, Garvin JH, Jr., Goldman S*, et al.* High dose chemotherapy with autologous bone marrow rescue for children with diffuse pontine brain stem tumors. Children's Cancer Group. J Neurooncol 1998;37(1):67-73.

18. Bouffet E, Raquin M, Doz F*, et al.* Radiotherapy followed by high dose busulfan and thiotepa: a prospective assessment of high dose chemotherapy in children with diffuse pontine gliomas. Cancer 2000;88(3):685-92.
19. Marks P, Rifkind RA, Richon VM, Breslow R, Miller T, Kelly WK. Histone deacetylases and cancer: causes and therapies. Nat Rev Cancer 2001;1(3):194-202.
20. Marks PA, Dokmanovic M. Histone deacetylase inhibitors: discovery and development as anticancer agents. Expert Opin Investig Drugs 2005;14(12):1497-511.
21. Roth SY, Denu JM, Allis CD. Histone acetyltransferases. Annu Rev Biochem 2001;70:81-120.
22. de Ruijter AJ, van Gennip AH, Caron HN, Kemp S, van Kuilenburg AB. Histone deacetylases (HDACs): characterization of the classical HDAC family. Biochem J 2003;370(Pt 3):737-49.
23. Fraga MF, Ballestar E, Villar-Garea A*, et al.* Loss of acetylation at Lys16 and trimethylation at Lys20 of histone H4 is a common hallmark of human cancer. Nat Genet 2005;37(4):391-400.
24. Blaheta RA, Michaelis M, Driever PH, Cinatl J, Jr. Evolving anticancer drug valproic acid: insights into the mechanism and clinical studies. Med Res Rev 2005;25(4):383-97.
25. Li XN, Shu Q, Su JM, Perlaky L, Blaney SM, Lau CC. Valproic acid induces growth arrest, apoptosis, and senescence in medulloblastomas by increasing histone hyperacetylation and regulating expression of p21Cip1, CDK4, and CMYC. Mol Cancer Ther 2005;4(12):1912-22.
26. Shu Q, Antalffy B, Su JM*, et al.* Valproic Acid prolongs survival time of severe combined immunodeficient mice bearing intracerebellar orthotopic medulloblastoma xenografts. Clin Cancer Res 2006;12(15):4687-94.
27. Eyupoglu IY, Hahnen E, Buslei R*, et al.* Suberoylanilide hydroxamic acid (SAHA) has potent anti-glioma properties in vitro, ex vivo and in vivo. J Neurochem 2005;93(4):992-9.
28. Knupfer MM, Hernaiz-Driever P, Poppenborg H, Wolff JE, Cinatl J. Valproic acid inhibits proliferation and changes expression of CD44 and CD56 of malignant glioma cells in vitro. Anticancer Res 1998;18(5A):3585-9.
29. Knupfer MM, Pulzer F, Schindler I, Hernaiz Driever P, Knupfer H, Keller E. Different effects of valproic acid on proliferation and migration of malignant glioma cells in vitro. Anticancer Res 2001;21(1A):347-51.
30. Chavez-Blanco A, Perez-Plasencia C, Perez-Cardenas E*, et al.* Antineoplastic effects of the DNA methylation inhibitor hydralazine and the histone deacetylase inhibitor valproic acid in cancer cell lines. Cancer Cell Int 2006;6:2.
31. Das CM, Aguilera D, Vasquez H*, et al.* Valproic acid induces p21 and topoisomerase-II (alpha/beta) expression and synergistically enhances etoposide cytotoxicity in human glioblastoma cell lines. J Neurooncol 2007;85(2):159-70.
32. Benitez JA, Arregui L, Cabrera G, Segovia J. Valproic acid induces polarization, neuronal-like differentiation of a subpopulation of C6 glioma cells and selectively regulates transgene expression. Neuroscience 2008.
33. Eyupoglu IY, Hahnen E, Trankle C*, et al.* Experimental therapy of malignant gliomas using the inhibitor of histone deacetylase MS-275. Mol Cancer Ther 2006;5(5):1248-55.
34. Wetzel M, Premkumar DR, Arnold B, Pollack IF. Effect of trichostatin A, a histone deacetylase inhibitor, on glioma proliferation in vitro by inducing cell cycle arrest and apoptosis. J Neurosurg 2005;103(6 Suppl):549-56.
35. Yin D, Ong JM, Hu J*, et al.* Suberoylanilide hydroxamic acid, a histone deacetylase inhibitor: effects on gene expression and growth of glioma cells in vitro and in vivo. Clin Cancer Res 2007;13(3):1045-52.

36. Kamitani H, Taniura S, Watanabe K, Sakamoto M, Watanabe T, Eling T. Histone acetylation may suppress human glioma cell proliferation when p21 WAF/Cip1 and gelsolin are induced. Neuro Oncol 2002;4(2):95-101.
37. Oberndorfer S, Piribauer M, Marosi C, Lahrmann H, Hitzenberger P, Grisold W. P450 enzyme inducing and non-enzyme inducing antiepileptics in glioblastoma patients treated with standard chemotherapy. J Neurooncol 2005;72(3):255-60.
38. Witt O, Schweigerer L, Driever PH, Wolff J, Pekrun A. Valproic acid treatment of glioblastoma multiforme in a child. Pediatr Blood Cancer 2004;43(2):181.
39. Wolff JE, Kramm C, Kortmann RD*, et al.* Valproic acid was well tolerated in heavily pretreated pediatric patients with high-grade glioma. J Neurooncol 2008.
40. Chinnaiyan P, Cerna D, Burgan WE*, et al.* Postradiation sensitization of the histone deacetylase inhibitor valproic Acid. Clin Cancer Res 2008;14(17):5410-5.
41. Camphausen K, Cerna D, Scott T*, et al.* Enhancement of in vitro and in vivo tumor cell radiosensitivity by valproic acid. Int J Cancer 2005;114(3):380-6.
42. Harikrishnan KN, Karagiannis TC, Chow MZ, El-Osta A. Effect of valproic acid on radiation-induced DNA damage in euchromatic and heterochromatic compartments. Cell Cycle 2008;7(4):468-76.
43. Karagiannis TC, Kn H, El-Osta A. The epigenetic modifier, valproic acid, enhances radiation sensitivity. Epigenetics 2006;1(3):131-7.
44. Kim JH, Shin JH, Kim IH. Susceptibility and radiosensitization of human glioblastoma cells to trichostatin A, a histone deacetylase inhibitor. Int J Radiat Oncol Biol Phys 2004;59(4):1174-80.
45. Entin-Meer M, Rephaeli A, Yang X, Nudelman A, VandenBerg SR, Haas-Kogan DA. Butyric acid prodrugs are histone deacetylase inhibitors that show antineoplastic activity and radiosensitizing capacity in the treatment of malignant gliomas. Mol Cancer Ther 2005;4(12):1952-61.
46. Camphausen K, Burgan W, Cerra M*, et al.* Enhanced radiation-induced cell killing and prolongation of gammaH2AX foci expression by the histone deacetylase inhibitor MS-275. Cancer Res 2004;64(1):316-21.
47. Sonnemann J, Kumar KS, Heesch S*, et al.* Histone deacetylase inhibitors induce cell death and enhance the susceptibility to ionizing radiation, etoposide, and TRAIL in medulloblastoma cells. Int J Oncol 2006;28(3):755-66.
48. Chinnaiyan P, Vallabhaneni G, Armstrong E, Huang SM, Harari PM. Modulation of radiation response by histone deacetylase inhibition. Int J Radiat Oncol Biol Phys 2005;62(1):223-9.
49. Munshi A, Tanaka T, Hobbs ML, Tucker SL, Richon VM, Meyn RE. Vorinostat, a histone deacetylase inhibitor, enhances the response of human tumor cells to ionizing radiation through prolongation of gamma-H2AX foci. Mol Cancer Ther 2006;5(8):1967-74.
50. Entin-Meer M, Yang X, VandenBerg SR*, et al.* In vivo efficacy of a novel histone deacetylase inhibitor in combination with radiation for the treatment of gliomas. Neuro Oncol 2007;9(2):82-8.
51. Zgouras D, Becker U, Loitsch S, Stein J. Modulation of angiogenesis-related protein synthesis by valproic acid. Biochem Biophys Res Commun 2004;316(3):693-7.
52. Isenberg JS, Jia Y, Field L*, et al.* Modulation of angiogenesis by dithiolethione-modified NSAIDs and valproic acid. Br J Pharmacol 2007;151(1):63-72.
53. Gao D, Xia Q, Lv J, Zhang H. Chronic administration of valproic acid inhibits PC3 cell growth by suppressing tumor angiogenesis in vivo. Int J Urol 2007;14(9):838-45.
54. Shabbeer S, Kortenhorst MS, Kachhap S, Galloway N, Rodriguez R, Carducci MA. Multiple Molecular pathways explain the anti-proliferative effect of valproic acid on prostate cancer cells in vitro and in vivo. Prostate 2007;67(10):1099-110.

55. Shen S, Li J, Casaccia-Bonnefil P. Histone modifications affect timing of oligodendrocyte progenitor differentiation in the developing rat brain. J Cell Biol 2005;169(4):577-89.
56. Balasubramaniyan V, Boddeke E, Bakels R*, et al.* Effects of histone deacetylation inhibition on neuronal differentiation of embryonic mouse neural stem cells. Neuroscience 2006;143(4):939-51.
57. Hao Y, Creson T, Zhang L*, et al.* Mood stabilizer valproate promotes ERK pathway-dependent cortical neuronal growth and neurogenesis. J Neurosci 2004;24(29):6590-9.
58. Hsieh J, Nakashima K, Kuwabara T, Mejia E, Gage FH. Histone deacetylase inhibition-mediated neuronal differentiation of multipotent adult neural progenitor cells. Proc Natl Acad Sci U S A 2004;101(47):16659-64.
59. Bug G, Gul H, Schwarz K*, et al.* Valproic acid stimulates proliferation and self-renewal of hematopoietic stem cells. Cancer Res 2005;65(7):2537-41.
60. De Felice L, Tatarelli C, Mascolo MG*, et al.* Histone deacetylase inhibitor valproic acid enhances the cytokine-induced expansion of human hematopoietic stem cells. Cancer Res 2005;65(4):1505-13.
61. Cho HH, Park HT, Kim YJ, Bae YC, Suh KT, Jung JS. Induction of osteogenic differentiation of human mesenchymal stem cells by histone deacetylase inhibitors. J Cell Biochem 2005;96(3):533-42.
62. Cimino G, Lo-Coco F, Fenu S*, et al.* Sequential valproic acid/all-trans retinoic acid treatment reprograms differentiation in refractory and high-risk acute myeloid leukemia. Cancer Res 2006;66(17):8903-11.
63. Cheng YC, Lin H, Huang MJ, Chow JM, Lin S, Liu HE. Downregulation of c-Myc is critical for valproic acid-induced growth arrest and myeloid differentiation of acute myeloid leukemia. Leuk Res 2007;31(10):1403-11.
64. Charalambous C, Hofman FM, Chen TC. Functional and phenotypic differences between glioblastoma multiforme-derived and normal human brain endothelial cells. J Neurosurg 2005;102(4):699-705.
65. Huang H, Held-Feindt J, Buhl R, Mehdorn HM, Mentlein R. Expression of VEGF and its receptors in different brain tumors. Neurol Res 2005;27(4):371-7.
66. Jain R, Scarpace L, Ellika S*, et al.* First-pass perfusion computed tomography: initial experience in differentiating recurrent brain tumors from radiation effects and radiation necrosis. Neurosurgery 2007;61(4):778-86; discussion 86-7.
67. Yuan F, Chen Y, Dellian M, Safabakhsh N, Ferrara N, Jain RK. Time-dependent vascular regression and permeability changes in established human tumor xenografts induced by an anti-vascular endothelial growth factor/vascular permeability factor antibody. Proc Natl Acad Sci U S A 1996;93(25):14765-70.
68. Gordon MS, Margolin K, Talpaz M*, et al.* Phase I safety and pharmacokinetic study of recombinant human anti-vascular endothelial growth factor in patients with advanced cancer. J Clin Oncol 2001;19(3):843-50.
69. Margolin K, Gordon MS, Holmgren E*, et al.* Phase Ib trial of intravenous recombinant humanized monoclonal antibody to vascular endothelial growth factor in combination with chemotherapy in patients with advanced cancer: pharmacologic and long-term safety data. J Clin Oncol 2001;19(3):851-6.
70. Kabbinavar F, Hurwitz HI, Fehrenbacher L*, et al.* Phase II, randomized trial comparing bevacizumab plus fluorouracil (FU)/leucovorin (LV) with FU/LV alone in patients with metastatic colorectal cancer. J Clin Oncol 2003;21(1):60-5.

71. Yang JC, Haworth L, Sherry RM*, et al.* A randomized trial of bevacizumab, an anti-vascular endothelial growth factor antibody, for metastatic renal cancer. N Engl J Med 2003;349(5):427-34.
72. Vredenburgh JJ, Desjardins A, Herndon JE, 2nd*, et al.* Phase II trial of bevacizumab and irinotecan in recurrent malignant glioma. Clin Cancer Res 2007;13(4):1253-9.
73. Vredenburgh JJ, Desjardins A, Herndon JE, 2nd*, et al.* Bevacizumab plus irinotecan in recurrent glioblastoma multiforme. J Clin Oncol 2007;25(30):4722-9.
74. Cloughesy TF, Prados MD, Wen PY*, et al.* A phase II, randomized, non-comparative clinical trial of the effect of bevacizumab (BV) alone or in combination with irinotecan (CPT) on 6-month progression free survival (PFS6) in recurrent, treatment-refractory glioblastoma (GBM). J Clin Oncol 2008;26(May 20 suppl; abstr 2010b).
75. Friedman HS, Petros WP, Friedman AH*, et al.* Irinotecan therapy in adults with recurrent or progressive malignant glioma. J Clin Oncol 1999;17(5):1516-25.
76. Cloughesy TF, Filka E, Kuhn J*, et al.* Two studies evaluating irinotecan treatment for recurrent malignant glioma using an every-3-week regimen. Cancer 2003;97(9 Suppl):2381-6.
77. Chamberlain MC. Salvage chemotherapy with CPT-11 for recurrent glioblastoma multiforme. J Neurooncol 2002;56(2):183-8.
78. Prados MD, Lamborn K, Yung WK*, et al.* A phase 2 trial of irinotecan (CPT-11) in patients with recurrent malignant glioma: a North American Brain Tumor Consortium study. Neuro Oncol 2006;8(2):189-93.
79. Batchelor TT, Gilbert MR, Supko JG*, et al.* Phase 2 study of weekly irinotecan in adults with recurrent malignant glioma: final report of NABTT 97-11. Neuro Oncol 2004;6(1):21-7.
80. Buckner JC, Reid JM, Wright K*, et al.* Irinotecan in the treatment of glioma patients: current and future studies of the North Central Cancer Treatment Group. Cancer 2003;97(9 Suppl):2352-8.
81. Raymond E, Fabbro M, Boige V*, et al.* Multicentre phase II study and pharmacokinetic analysis of irinotecan in chemotherapy-naive patients with glioblastoma. Ann Oncol 2003;14(4):603-14.
82. Reardon DA, Quinn JA, Rich JN*, et al.* Phase I trial of irinotecan plus temozolomide in adults with recurrent malignant glioma. Cancer 2005;104(7):1478-86.
83. Loghin ME, Prados MD, Wen P*, et al.* Phase I study of temozolomide and irinotecan for recurrent malignant gliomas in patients receiving enzyme-inducing antiepileptic drugs: a north american brain tumor consortium study. Clin Cancer Res 2007;13(23):7133-8.
84. Reardon DA, Quinn JA, Rich JN*, et al.* Phase 2 trial of BCNU plus irinotecan in adults with malignant glioma. Neuro Oncol 2004;6(2):134-44.
85. Bender JL, Adamson PC, Reid JM*, et al.* Phase I trial and pharmacokinetic study of bevacizumab in pediatric patients with refractory solid tumors: a Children's Oncology Group Study. J Clin Oncol 2008;26(3):399-405.
86. Loscher W. Valproate: a reappraisal of its pharmacodynamic properties and mechanisms of action. Prog Neurobiol 1999;58(1):31-59.
87. Lindberger M, Tomson T, Wallstedt L, Stahle L. Distribution of valproate to subdural cerebrospinal fluid, subcutaneous extracellular fluid, and plasma in humans: a microdialysis study. Epilepsia 2001;42(2):256-61.
88. Hegi ME, Diserens AC, Gorlia T*, et al.* MGMT gene silencing and benefit from temozolomide in glioblastoma. N Engl J Med 2005;352(10):997-1003.
89. Sathornsumetee S, Cao Y, Marcello JE*, et al.* Tumor angiogenic and hypoxic profiles predict radiographic response and survival in malignant astrocytoma patients treated with bevacizumab and irinotecan. J Clin Oncol 2008;26(2):271-8.

90. Schreiber V, Dantzer F, Ame JC, de Murcia G. Poly(ADP-ribose): novel functions for an old molecule. Nat Rev Mol Cell Biol 2006;7(7):517-28.
91. Marchion DC, Bicaku E, Daud AI, al e. Valproic acid alters chromatin structure by regulation of chromatin modulation proteins. Cancer Res 2005;65:3815-22.
92. Marchion DC, Bicaku E, Turner JG, al e. HDAC2 regulates chromatin plasticity and enhances DNA vulnerability. Mol Cancer Ther 2009;8:794-801.
93. Kim MS, Blake M, Baek JH, al e. Inhibition of histone deacetylase increases cytotoxicity to anticancer drugs targeting DNA. Cancer Res 2003;63:7291-300.
94. Marchion DC, Bicaku E, Daud AI, al e. Sequence-specific potentiation of topoisomerase II inhibitors by the histone deacetylase inhibitor suberoylanilide hydroxamic acid. J Cell Biochem 2004;92:223-37.
95. Marquard L, Gjerdrum LM, Christensen IJ, al e. Prognostic significance of the therapeutic targets histone deacetylase 1, 2, 6 and acetylated histone H4 in cutaneous T-cell lymphoma. Histopathology 2008;53:267-77.
96. Munster P, Marchion D, Bicaku E, al e. Phase I trial of histone deacetylase inhibition by valproic acid followed by the topoisomerase II inhibitor epirubicin in advanced solid tumors: a clinical and translational study. J Clin Oncol 2007;25:1979-85.
97. Munster P, Marchion D, Bicaku E, al e. Clinical and biological effects of valproic acid as a histone deacetylase inhibitor on tumor and surrogate tissues: phase I/II trial of valproic acid and epirubicin/FEC. Clin Cancer Res 2009;15:2488-96.
98. Friedman HS, Prados MD, Wen PY*, et al.* Bevacizumab alone and in combination with irinotecan in recurrent glioblastoma. J Clin Oncol 2009;27(28):4733-40.
99. Vredenburgh JJ, Desjardins A, Kirkpatrick JP*, et al.* Addition of bevacizumab to standard radiation therapy and daily temozolomide is associated with minimal toxicity in newly diagnosed glioblastoma multiforme. Int J Radiat Oncol Biol Phys;82(1):58-66.
100. Acharya S, Bussel JB. Hematologic toxicity of sodium valproate. J Pediatr Hematol Oncol 2000;22(1):62-5.
101. Delgado MR, Riela AR, Mills J, Browne R, Roach ES. Thrombocytopenia secondary to high valproate levels in children with epilepsy. J Child Neurol 1994;9(3):311-4.
102. Ko CH, Kong CK, Tse PW. Valproic acid and thrombocytopenia: cross-sectional study. Hong Kong Med J 2001;7(1):15-21.
103. Henriksen O, Johannessen SI. Clinical and pharmacokinetic observations on sodium valproate - a 5-year follow-up study in 100 children with epilepsy. Acta Neurol Scand 1982;65(5):504-23.
104. Allarakhia IN, Garofalo EA, Komarynski MA, Robertson PL. Valproic acid and thrombocytopenia in children: a case-controlled retrospective study. Pediatr Neurol 1996;14(4):303-7.
105. Verrotti A, Greco R, Matera V, Altobelli E, Morgese G, Chiarelli F. Platelet count and function in children receiving sodium valproate. Pediatr Neurol 1999;21(3):611-4.
106. Bryant AE, 3rd, Dreifuss FE. Valproic acid hepatic fatalities. III. U.S. experience since 1986. Neurology 1996;46(2):465-9.
107. Koenig SA, Buesing D, Longin E*, et al.* Valproic acid-induced hepatopathy: nine new fatalities in Germany from 1994 to 2003. Epilepsia 2006;47(12):2027-31.
108. Verrotti A, Trotta D, Morgese G, Chiarelli F. Valproate-induced hyperammonemic encephalopathy. Metab Brain Dis 2002;17(4):367-73.
109. Bohan TP, Helton E, McDonald I*, et al.* Effect of L-carnitine treatment for valproate-induced hepatotoxicity. Neurology 2001;56(10):1405-9.

110. De Vivo DC, Bohan TP, Coulter DL*, et al.* L-carnitine supplementation in childhood epilepsy: current perspectives. Epilepsia 1998;39(11):1216-25.
111. Grauso-Eby NL, Goldfarb O, Feldman-Winter LB, McAbee GN. Acute pancreatitis in children from Valproic acid: case series and review. Pediatr Neurol 2003;28(2):145-8.
112. Glusker P, Recht L, Lane B. Reversible posterior leukoencephalopathy syndrome and bevacizumab. N Engl J Med 2006;354(9):980-2; discussion -2.
113. Ozcan C, Wong SJ, Hari P. Reversible posterior leukoencephalopathy syndrome and bevacizumab. N Engl J Med 2006;354(9):980-2; discussion -2.
114. Miller KD, Chap LI, Holmes FA*, et al.* Randomized phase III trial of capecitabine compared with bevacizumab plus capecitabine in patients with previously treated metastatic breast cancer. J Clin Oncol 2005;23(4):792-9.
115. Sandler A, Gray R, Perry MC*, et al.* Paclitaxel-carboplatin alone or with bevacizumab for non-small-cell lung cancer. N Engl J Med 2006;355(24):2542-50.
116. Gerber HP, Vu TH, Ryan AM, Kowalski J, Werb Z, Ferrara N. VEGF couples hypertrophic cartilage remodeling, ossification and angiogenesis during endochondral bone formation. Nat Med 1999;5(6):623-8.
117. Ryan AM, Eppler DB, Hagler KE*, et al.* Preclinical safety evaluation of rhuMAbVEGF, an antiangiogenic humanized monoclonal antibody. Toxicol Pathol 1999;27(1):78-86.
118. Smith AR, Hennessy JM, Kurth MA, Nelson SC. Reversible skeletal changes after treatment with bevacizumab in a child with cutaneovisceral angiomatosis with thrombocytopenia syndrome. Pediatr Blood Cancer 2008;51(3):418-20.
119. Smeaton MB, Miller PS, Ketner G, Hanakahi LA. Small-scale extracts for the study of nucleotide excision repair and non-homologous end joining. Nucleic Acids Res 2007;35(22):e152.
120. Woolson RF. Rank tests and a one-sample logrank test for comparing observed data to a standard population. Biometrics 1981;37:687-96.

APPENDIX IA
AVASTIN™ (BEVACIZUMAB) REQUEST FORM
Investigator Sponsored Trials

Complete and **FAX** this request form to **Tara McCartney, RPh, or Jennifer Lynds, RPh, Pharm. D.** at **Texas Children's Hospital Investigational Pharmacy Services (IPS), fax number 832-825-3901**. Study drug may be ordered Monday through Friday, from 8 am to 4 pm (Central Standard Time). **Please order study drug at least 5 days in advance.** Contact IPS at 832-822-3899 if you have any questions. *Please print all entries clearly.*

A Phase 2 Study of Valproic Acid and Radiation, Followed by Maintenance Valproic Acid and Bevacizumab in Children with Newly Diagnosed High-Grade Gliomas or Brainstem Gliomas	
Investigator:	Requestor:
Institution:	Phone Number:
Patient ID Number:	
Weight (kg): Height (cm): BSA (m^2):	

BEVACIZUMAB REQUEST FORM		
Weight (kg)	**Dose (mg) (10 mg/kg/dose)**	**400 mg vial (20 ml)**
0-10	0-100	1
10.1-20	101-200	1
20.1-30	201-300	1
30.1-40	301-400	1
40.1-50	401-500	2
50.1-60	501-600	2
60.1-70	601-700	2
70.1-80	701-800	2
80.1-90	801-900	3
90.1-100	901-1000	3
# of 400 mg vial _____ x 6 (12-week supply) = _____ 400-mg vials requested		

DELIVERY TO:
CONTACT:
PHONE:

Investigator's Signature _____ ***Date*** _____

APPENDIX IB
AVASTIN™ (BEVACIZUMAB) INVENTORY RECORD
Investigator Sponsored Trials

A Phase 2 Study of Valproic Acid and Radiation, Followed by Maintenance Valproic Acid and Bevacizumab in Children with Newly Diagnosed High-Grade Gliomas or Brainstem Gliomas	
Investigator:	Inventory Recorder:
Institution:	Phone Number:
Patient ID Number:	

BEVACIZUMAB INVENTORY RECORD					
Date Given	**Weight (kg)**	**Dose (mg)**	**400 mg Vials Started**	**400 mg Vials Used**	**400 mg Vials Remaining**

Please complete and fax Bevacizumab Inventory Record to Sue Burlingame (Phone: 832-824-1532; Fax: 832-825-1198) or Suzanne Wheeler (Phone: 832-824-4217; Fax 832-825-1198) every 12 weeks.

Investigator's Signature _____ **Date** _____

APPENDIX II

Procedure for Obtaining a Urine Protein / Creatinine Ratio

1) Obtain at least 4 ml of a random urine sample (does not have to be a 24 hour urine)
2) Determine protein concentration (mg/dL)
3) Determine creatinine concentration (mg/dL)
4) Urine protein / creatinine ratio = protein concentration (mg /dL) divide by creatinine concentration (mg /dL)

The UPC directly correlates with the amount of protein excreted in the urine per 24 hrs (i.e. a UPC of 1 should be equivalent to 1g protein in a 24hr urine collection)

Protein and creatinine concentrations should be available on standard reports of urinalyses, not dipsticks. If protein and creatinine concentrations are not routinely reported at an Institution, their measurements and reports may need to be requested.

APPENDIX III

90th and 95th Percentile Blood Pressure For Girls 1-17 Years

Age (y)	Blood Pressure Percentile	Systolic Blood Pressure by Percentile of Height (mm Hg)							Diastolic Blood Pressure by Percentile of Height (mm Hg)						
		5%	10%	25%	50%	75%	90%	95%	5%	10%	25%	50%	75%	90%	95%
1	90th	97	98	99	100	102	103	104	53	53	53	54	55	56	56
	95th	101	102	103	104	105	107	107	57	57	57	58	59	60	60
2	90th	99	99	100	102	103	104	105	57	57	58	58	59	60	61
	95th	102	103	104	105	107	108	109	61	61	62	62	63	64	65
3	90th	100	100	102	103	104	105	106	61	61	61	62	63	63	64
	95th	104	104	105	107	108	109	110	65	65	65	66	67	67	68
4	90th	101	102	103	104	106	107	108	63	63	64	65	65	66	67
	95th	105	106	107	108	109	111	111	67	67	68	69	69	70	71
5	90th	103	103	104	106	107	108	109	65	66	66	67	68	68	69
	95th	107	107	108	110	111	112	113	69	70	70	71	72	72	73
6	90th	104	105	106	107	109	110	111	67	67	68	69	69	70	71
	95th	108	109	110	111	112	114	114	71	71	72	73	73	74	75
7	90th	106	107	108	109	110	112	112	69	69	69	70	71	72	72
	95th	110	110	112	113	114	115	116	73	73	73	74	75	76	76
8	90th	108	109	110	111	112	113	114	70	70	71	71	72	73	74
	95th	112	112	113	115	116	117	118	74	74	75	75	76	77	78
9	90th	110	110	112	113	114	115	116	71	72	72	73	74	74	75
	95th	114	114	115	117	118	119	120	75	76	76	77	78	78	79
10	90th	112	112	114	115	116	117	118	73	73	73	74	75	76	76
	95th	116	116	117	119	120	121	122	77	77	77	78	79	80	80
11	90th	114	114	116	117	118	119	120	74	74	75	75	76	77	77
	95th	118	118	119	121	122	123	124	78	78	79	79	80	81	81
12	90th	116	116	118	119	120	121	122	75	75	76	76	77	78	78
	95th	120	120	121	123	124	125	126	79	79	80	80	81	82	82
13	90th	118	118	119	121	122	123	124	76	76	77	78	78	79	80
	95th	121	122	123	125	126	127	128	80	80	81	82	82	83	84
14	90th	119	120	121	122	124	125	126	77	77	78	79	79	80	81
	95th	123	124	125	126	128	129	130	81	81	82	83	83	84	85
15	90th	121	121	122	124	125	126	127	78	78	79	79	80	81	82
	95th	124	125	126	128	129	130	131	82	82	83	83	84	85	86
16	90th	122	122	123	125	126	127	128	79	79	79	80	81	82	82
	95th	125	126	127	128	130	131	132	83	83	83	84	85	86	86
17	90th	122	123	124	125	126	128	128	79	79	79	80	81	82	82
	95th	126	126	127	129	130	131	132	83	83	83	84	85	86	86

APPENDIX III

90th and 95th Percentile Blood Pressure For Boys 1-17 Years

Age (y)	Blood Pressure Percentile	Systolic Blood Pressure by Percentile of Height (mm Hg)							Diastolic Blood Pressure by Percentile of Height (mm Hg)						
		5%	10%	25%	50%	75%	90%	95%	5%	10%	25%	50%	75%	90%	95%
1	90th	94	95	97	98	100	102	102	50	51	52	53	54	54	55
	95th	98	99	101	102	104	106	106	55	55	56	57	58	59	59
2	90th	98	99	100	102	104	105	106	55	55	56	57	58	59	59
	95th	101	102	104	106	108	109	110	59	59	60	61	62	63	63
3	90th	100	101	103	105	107	108	109	59	59	60	61	62	63	63
	95th	104	105	107	109	111	112	113	63	63	64	65	66	67	67
4	90th	102	103	105	107	109	110	111	62	62	63	64	65	66	66
	95th	106	107	109	111	113	114	115	66	67	67	68	69	70	71
5	90th	104	105	106	108	110	112	112	65	65	66	67	68	69	69
	95th	108	109	110	112	114	115	116	69	70	70	71	72	73	74
6	90th	105	106	108	110	111	113	114	67	68	69	70	70	71	72
	95th	109	110	112	114	115	117	117	72	72	73	74	75	76	76
7	90th	106	107	109	111	113	114	115	69	70	71	72	72	73	74
	95th	110	111	113	115	116	118	119	74	74	75	76	77	78	78
8	90th	107	108	110	112	114	115	116	71	71	72	73	74	75	75
	95th	111	112	114	116	118	119	120	75	76	76	77	78	79	80
9	90th	109	110	112	113	115	117	117	72	73	73	74	75	76	77
	95th	113	114	116	117	119	121	121	76	77	78	79	80	80	81
10	90th	110	112	113	115	117	118	119	73	74	74	75	76	77	78
	95th	114	115	117	119	121	122	123	77	78	79	80	80	81	82
11	90th	112	113	115	117	119	120	121	74	74	75	76	77	78	78
	95th	116	117	119	121	123	124	125	78	79	79	80	81	82	83
12	90th	115	116	117	119	121	123	123	75	75	76	77	78	78	79
	95th	119	120	121	123	125	126	127	79	79	80	81	82	83	83
13	90th	117	118	120	122	124	125	126	75	76	76	77	78	79	80
	95th	121	122	124	126	128	129	130	79	80	81	82	83	83	84
14	90th	120	121	123	125	126	128	128	76	76	77	78	79	80	80
	95th	124	125	127	128	130	132	132	80	81	81	82	83	84	85
15	90th	123	124	125	127	129	131	131	77	77	78	79	80	81	81
	95th	127	128	129	131	133	134	135	81	82	83	83	84	85	86
16	90th	125	126	128	130	132	133	134	79	79	80	81	82	82	83
	95th	129	130	132	134	136	137	138	83	83	84	85	86	87	87
17	90th	128	129	131	133	134	136	136	81	81	82	83	84	85	85
	95th	132	133	135	136	138	140	140	85	85	86	87	88	89	89

APPENDIX IV: VALPROIC ACID ADMINISTRATION DIARY DURING RADIATION, POST XRT, AND MAINTAINANCE THERAPY

Patient initials: _____ Patient ID: _____ Institution: _____

1) Circle name of drug given (Depakene, Depakote, or Valproic Acid)
2) Record the date and time for each dose (circle AM or PM). Please try to adhere to a strict schedule of giving the drug every 8 hours.
3) Record the number of Depakene, Depakote, or Valproic Acid capsules given or the amount of syrup in milliliters (ml) given.
4) The capsules can be swallowed whole without breakage, or alternatively, the content of the capsule can be sprinkled and mixed with water, juice, or food. Syrup can be mixed with water, juice, or food.

Example: Drug given: Depakene, Depakote, or Valproic Acid

DAY	DATE	MORNING	AFTERNOON	EVENING	NUMBER OF CAPSULES?		AMOUNT GIVEN?	COMMENTS
					125 mg	250 mg	ML	
1	*9/12/09*	*7 : 10* AM PM	*3 : 20* AM PM	*11 :00* AM PM	*2*			*He felt nauseated after taking pills.*

Week # _____ Drug given: Depakene, Depakote, or Valproic Acid

DAY	DATE	MORNING	AFTERNOON	EVENING	NUMBER OF CAPSULES?		AMOUNT SYRUP?	COMMENTS
					125 mg	250 mg	ML	
1		: AM PM	: AM PM	: AM PM				
2		: AM PM	: AM PM	: AM PM				
3		: AM PM	: AM PM	: AM PM				
4		: AM PM	: AM PM	: AM PM				
5		: AM PM	: AM PM	: AM PM				
6		: AM PM	: AM PM	: AM PM				
7		: AM PM	: AM PM	: AM PM				

Week # _____ Drug given: Depakene, Depakote, or Valproic Acid

DAY	DATE	MORNING	AFTERNOON	EVENING	NUMBER OF CAPSULES?		AMOUNT SYRUP?	COMMENTS
					125 mg	250 mg	ML	
1		: AM PM	: AM PM	: AM PM				
2		: AM PM	: AM PM	: AM PM				
3		: AM PM	: AM PM	: AM PM				
4		: AM PM	: AM PM	: AM PM				
5		: AM PM	: AM PM	: AM PM				
6		: AM PM	: AM PM	: AM PM				
7		: AM PM	: AM PM	: AM PM				

This patient diary has been verified for accuracy by _____ on ____ / ____ / ____

APPENDIX V: COMPREHENSIVE ADVERSE EVENTS AND POTENTIAL RISK LISTS FOR BEVACIZUMAB

Category (Body System)	Adverse Events with Possible Relationship to Bevacizumab (CTCAE v3.0 Term)	'Agent Specific Adverse Event List' (ASAEL)
ALLERGY/IMMUNOLOGY		
	Allergic reaction/hypersensitivity (including drug fever)	***Allergic reaction/hypersensitivity (including drug fever)***
	Allergic rhinitis (including sneezing, nasal stuffiness, postnasal drip)	***Allergic rhinitis (including sneezing, nasal stuffiness, postnasal drip)***
BLOOD/BONE MARROW		
	Hemoglobin	***Hemoglobin***
	Leukocytes (total WBC)	***Leukocytes (total WBC)***
	Neutrophils/granulocytes (ANC/AGC)	***Neutrophils/granulocytes (ANC/AGC)***
CARDIAC ARRHYTHMIA		
	Supraventricular arrhythmia NOS	***Supraventricular arrhythmia NOS***
	Ventricular fibrillation	
CARDIAC GENERAL		
	Cardiac ischemia/infarction	***Cardiac ischemia/infarction***
	Cardiac troponin I (cTnl)	
	Hypertension	***Hypertension***
	Hypotension	
	Left ventricular diastolic dysfunction	
	Left ventricular systolic dysfunction	
CONSTITUTIONAL SYMPTOMS		
	Fatigue (asthenia, lethargy, malaise)	***Fatigue (asthenia, lethargy, malaise)***
	Fever (in the absence of neutropenia, where neutropenia is defined as ANC < 1.0 x 10^9/L)	***Fever (in the absence of neutropenia, where neutropenia is defined as ANC < 1.0 x 10^9/L)***
	Rigors/chills	***Rigors/chills***
	Weight loss	
DERMATOLOGY/SKIN		
	Pruritus/itching	***Pruritus/itching***
	Rash/desquamation	***Rash/desquamation***
	Ulceration	
	Urticaria (hives, welts, wheals)	***Urticaria (hives, welts, wheals)***
	Wound complication, non-infectious	
GASTROINTESTINAL		
	Anorexia	***Anorexia***

Category (Body System)	Adverse Events with Possible Relationship to Bevacizumab (CTCAE v3.0 Term)	'Agent Specific Adverse Event List' (ASAEL)
	Colitis	
	Constipation	***Constipation***
	Diarrhea	***Diarrhea***
	Fistula, GI – Select	
	Heartburn/dyspepsia	***Heartburn/dyspepsia***
	Ileus (functional obstruction of bowel, i.e., neuroconstipation)	
	Leak (including anastomotic), GI: large bowel	
	Mucositis/stomatitis (functional/symptomatic) – Select	***Mucositis/stomatitis (functional/symptomatic) – Select***
	Nausea	***Nausea***
	Perforation, GI – Select	
	Ulcer, GI – Select	
	Vomiting	***Vomiting***
HEMORRHAGE/BLEEDING		
	Hemorrhage, GI – Select	***Hemorrhage GI – Select***
	Hemorrhage, CNS	***Hemorrhage, CNS***
	Hemorrhage, GU: vagina	***Hemorrhage, GU: vagina***
	Hemorrhage, pulmonary/upper respiratory: lung	***Hemorrhage, pulmonary/upper respirator: lung***
	Hemorrhage, pulmonary/upper respiratory: nose	***Hemorrhage, pulmonary/upper respiratory: nose***
INFECTION		
	Infection with normal ANC or Grade 1 or 2 neutrophils – Select	
	Infection with normal ANC or Grade 1 or 2 neutrophils – Select (pelvis, peritoneal cavity, rectum, scrotum, skin, wound)	
METABOLIC/LABORATORY		
	Alkaline phosphatase	***Alkaline phosphatase***
	ALT, SGPT (serum glutamic pyruvic transaminase)	***ALT, SGPT (serum glutamic pyruvic transaminase)***
	AST, SGOT (serum glutamic oxaloacetic transaminase)	***AST, SGOT (serum glutamic oxaloacetic transaminase)***
	Bilirubin (hyperbilirubinemia)	***Bilirubin (hyperbilirubinemia)***
	Creatinine	
	Proteinuria	***Proteinuria***
NEUROLOGY		
	CNS cerebrovascular ischemia	***CNS cerebrovascular ischemia***
	Dizziness	***Dizziness***

Category (Body System)	Adverse Events with Possible Relationship to Bevacizumab (CTCAE v3.0 Term)	'Agent Specific Adverse Event List' (ASAEL)
	Neurology – Other: (Leukoencephalopathy syndrome including reversible posterior leukoencephalopathy syndrome [RPLS])	
PAIN		
	Pain – abdomen NOS	***Pain – abdomen NOS***
	Pain – chest/thorax NOS	***Pain – chest/thorax NOS***
	Pain – head/headache	***Pain – head/headache***
	Pain – joint	***Pain – joint***
	Pain - muscle	
	Pain – NOS	
PULMONARY/UPPER RESPIRATORY		
	Bronchospasm, wheezing	
	Cough	***Cough***
	Dyspnea (shortness of breath)	***Dyspnea (shortness of breath)***
	Fistula, pulmonary/upper respiratory – Select	
	Nasal cavity/paranasal sinus reactions	***Nasal cavity/paranasal sinus reactions***
	Voice changes/dysarthria (e.g., hoarseness, loss or alteration in voice, laryngitis)	***Voice changes/dysarthria (e.g., hoarseness, loss or alteration in voice, laryngitis)***
	Pulmonary/Upper Respiratory – Other (nasal-septal perforation)	
RENAL/GENITOURINARY		
	Fistula, GU – Select	
	Renal failure	
SYNDROMES		
	Cytokine release syndrome/acute infusion reaction	***Cytokine release syndrome/acute infusion reaction***
VASCULAR		
	Thrombosis/thrombus/embolism	***Thrombosis/thrombus/embolism***
	Visceral arterial ischemia (non-myocardial)	

APPENDIX VI: TUMOR SPECIMEN & CORRELATIVE STUDY TRANSMITTAL FORM

This completed form should be sent with each shipment of tumor specimen, tumor slides, or blood samples for correlative studies.

Institution Name:	
Institution Address:	
Patient initials:	**Patient ID #:**
Contact person:	**Phone number:**
Date of Surgery/Biopsy/Collection:	**Date of Shipment:**

☐ **FRESH FROZEN TUMOR MATERIAL**

A minimum of 250 mg (wet weight prior to being frozen in liquid nitrogen; approximately 1.5 x 1.5 x 1.5 cm of tumor sample) of tumor specimen is preferred. In patients whose tumors are only biopsied, submission of as much frozen tumor specimen as possible is strongly encouraged. **Frozen tumors should be shipped on dry ice via over-night express delivery on Monday through Thursday. If a tumor is resected on Friday or over the weekend, the frozen specimen should be kept at –80^{0}C over the weekend and shipped on Monday.** Please include a pathology report if available.

☐ **TUMOR SLIDES**

Ten to twelve unstained PLUS glass slides, containing 5-micron thick tumor sections from FFPE tumor blocks, should be shipped at room temperature.

☐ **BLOOD SAMPLES**

Whole blood samples (a minimum of 5-10 ml for children < 12 kg, and 10-20 ml for children ≥ 12 kg) should be collected in either **heparinized (green tops, either sodium or lithium heparin is acceptable) or ACDA (yellow top) tubes.** ACDA tubes are preferred if available. **DO NOT collect blood samples on a Friday.**

- Seal the original tube(s) with paraffin and place the tube(s) inside an insulated container
- Place the container in a Styrofoam box.
- Between April-October: please ship with an ice pack placed in the Styrofoam box (ice pack should not be placed directly next to the samples). An ice pack is not necessary when shipping between November-March.
- Package sample as appropriate for biologic material

PBMC Collection	Date Collected
Prior to first dose of VPA	
During week 3 of VPA and XRT	

PLEASE SEND ALL SAMPLES TO THE FOLLOWING ADDRESS

Jack Su, MD
Attention: Elizabeth Hinojosa
Feigin Center, Baylor College of Medicine
1102 Bates Street, Rm 1030
Houston, Texas 77030
(832) 824-4688 or (832) 822-4306

Prior to shipping, please e-mail Dr. Jack Su (jmsu@txccc.org), Dr. Li (xxli@txccc.org), Elizabeth Hinojosa (ERHINOJO@txccc.org) **and** Suzanne Wheeler (shwheele@txccc.org) for notification of sample shipment.

The site personnel who shipped the samples must sign and date this form below:

Name (print):_____ Date:_____

Phone number: _____

Appendix VII: Protocol Roadmap
(Pre-XRT, XRT and Post XRT Phases - Weeks 1-10)

Patient Initials: _____ Patient ID: _____

		Week #	Date	*VPA dose (mg/kg/day)	*Patient's weight (kg)	Assessments
Pre-XRT, XRT Phase and Post XRT	XRT Phase	Week 1				**Pre-XRT – Study Entry:** ▪ History, physical & neuro exam ▪ Vital signs, performance status, height, weight, BSA, blood pressure ▪ Labs: CBC, differential, platelets, chem. 7, albumin, ALT, AST, bilirubin, amylase, lipase, urine protein/CR (3), PT, PTT, INR, pregnancy test (4) ▪ CSF cytology (5) ▪ Valproic acid levels (VPA) (2, 6) ▪ If consented, correlative biology studies (7) ▪ MRI brain and spine (8,9) **XRT week 3** ▪ If consented, correlative biology studies (7) **XRT Phase (q week)** ▪ History, physical, neuro exam ▪ Vital signs, performance status, height, weight, BSA, blood pressure ▪ Labs (2): CBC, differential, platelets, ALT, AST, bilirubin, Valproic acid levels (VPA) (2, 6) ▪ Check patient diary & roadmap and fax weekly (10) **Post-XRT (q 2 weeks)** ▪ History, physical & neuro exam ▪ Vital signs, performance status, height, weight, BSA, blood pressure ▪ Labs (2): CBC, differential, platelets, ALT, AST, bilirubin, Valproic acid levels (VPA) (2, 6) ▪ Check patient diary (10) ▪ Fax roadmap & patient diary after Post XRT **Week 10** ▪ MRI brain (8) and spine (9) ▪ CSF cytology (5) ▪ X-ray Right Knee
		Week 2				
		Week 3				
		Week 4				
		Week 5				
		Week 6				
	Post XRT	Week 7 (if needed)				
		Week 8				
		Week 9 (if needed)				
		Week 10				
Post XRT (XRT may continue into break if there were delays during week 1-6).						

*Valproic Acid (VPA) will be administered daily in three divided doses. See protocol 5.2 for VPA administration and monitoring guidelines and 5.4 for VPA dose modifications. Patient's weight should be the weight used to calculate the current VPA dose.

2) Patients with grade 4 neutropenia, anemia, and/or thrombocytopenia should have CBC checked twice weekly until the cytopenia(s) improves to grade 3 or better. Patients who require a dose escalation to maintain targeted trough concentration should also have trough VPA concentration, CBC, and liver functions measured every 3-5 days until target is reached.

3) See Appendix II for Urine/Protein Creatinine ratio.

4) For females of child-bearing potential

5) CSF cytology at the time of diagnosis and subsequently during protocol therapy should be performed if clinically indicated but is not required for study entry.

6) See Section 5.2.3 for monitoring valproic acid concentrations.
7) See section 7.0 for requirement and timing for correlative biology studies. Section 7.3.2 whole blood samples should be collected prior to starting VPA and radiation, and then at 2 weeks after starting VPA and radiation.
8) For patients with possible radiographic progression on week 10 MRI, the next MRI should be performed at week 18 (see section 8.3). If week 18 MRI shows stable disease or better compared to week 10 MRI, then patients will continue MRI every 12 weeks.
9) MRI of brain +/- spine must be obtained within 2 weeks prior to study registration/entry and must include gradient echo imaging (GRE) sequences for identifying CNS/intra-tumoral hemorrhage. For patients with high grade gliomas, an MRI of spine should be performed at the time of study entry, but subsequent MRI of spine should only be repeated as clinically indicated.
10) Medication diaries and roadmap should be checked and faxed weekly during radiation therapy. Post- XRT medication diaries and roadmap should be checked every 2 weeks and faxed every 4 weeks. Fax documents to (832)824-1198. Maintain copies of the patient's diary in the research chart.

Appendix VII: Protocol Roadmap (Maintenance Therapy – Weeks 11 - 34)

Patient Initials: _____ Patient ID: _____

	Week #	Date	^Bevacizumab (mg)	^Patient's weight (kg)	*VPA dose (mg/kg/day)	*Patient's weight (kg)	Assessments
Maintenance Therapy	Week 11						**Pre-Rx** ▪ History, physical & neuro exam ▪ Vital signs, performance status, height, weight, BSA, blood pressure ▪ Labs: CBC, differential, platelets (2), chem 7, albumin, ALT, AST, bilirubin, amylase, lipase, urine protein/CR (3), PT, PTT, INR, pregnancy test (4), Valproic acid levels (2, 6) ▪ X-ray of right knee (12) ▪ Fax roadmap and patient diary (13) **Q 2 weeks** ▪ Blood pressure ▪ Check patient diary (13) **Q 4 weeks** ▪ History, physical & neuro exam (1) ▪ Vital signs (1), performance status, height, weight, BSA, ▪ Labs: CBC, differential, platelets (2), chem 7, albumin; ALT, AST, bilirubin(2); urine protein/CR(3), Valproic acid levels (2, 6) ▪ MRI brain (10) ▪ Fax roadmap and patient diary (13) **Q 12 weeks** ▪ MRI brain (9) and spine (11) ▪ CSF cytology (5) **Q 24 weeks** ▪ X-ray of right knee (12)
	Week 12						
	Week 13						
	Week 14						
	Week 15						
	Week 16						
	Week 17						
	Week 18						
	Week 19						
	Week 20						
	Week 21						
	Week 22						
	Week 23						
	Week 24						
	Week 25						
	Week 26						
	Week 27						
	Week 28						
	Week 29						
	Week 30						
	Week 31						
	Week 32						
	Week 33						
	Week 34						

^ Bevacizumab will be administered during maintenance therapy every 2 weeks for a maximum of 24 months (including XRT). See protocol 5.3 for administration and monitoring guidelines and 5.5 for dose modifications.

* Valproic Acid (VPA) will be given daily in three divided doses. See section 5.2 for administration and monitoring guidelines and 5.4 for dose modifications.

1) For patients with newly identified punctuate hemorrhage on MRI, physical exam, neurological assessment, and performance status should be evaluated every 2 weeks at least twice to closely monitor for signs/symptoms of worsening hemorrhage.

2) Patients with grade 4 neutropenia, anemia, and/or thrombocytopenia should have CBC checked twice weekly until the cytopenia(s) improves to grade 3 or better. Patients who require a dose escalation to maintain targeted trough concentration should also have trough VPA, CBC, and liver functions measured every 3-5 days until target is reached.
3) See Appendix II for Urine/Protein Creatinine ratio.
4) For female patients of child-bearing potential
5) CSF cytology at the time of diagnosis and subsequently during protocol therapy should be performed as clinically indicated but is not required for study entry.
6) See Section 5.2.3 for monitoring valproic acid concentrations.
8) For patients with possible radiographic progression on week 10 MRI, the next MRI should be performed at week 18 (see section 8.3). If week 18 MRI shows stable disease or better compared to week 10 MRI, then patients will continue MRI every 12 weeks.
9) During maintenance therapy, MRI of brain, including GRE images, should be performed every 12 weeks until completion of protocol therapy.
10) For patients with newly identified punctate hemorrhage on MRI, the next MRI, including GRE images, should be repeated every 4 weeks at least twice to monitor possible progression of hemorrhage. In the absence of radiographic progression of hemorrhage, the frequency of MRI studies can then return to every 12 weeks.
11) For patients with high grade gliomas, an MRI of the spine should be performed at the time of study entry, but subsequent MRI of the spine should only be repeated as clinically indicated.
12) Pre-treatment tibial X-ray (AP and lateral views) of the right knee must be obtained in all patients younger than 16 years of age prior to starting maintenance therapy and be repeated every 24 weeks while receiving bevacizumab. If abnormalities of the metaphyseal plates are detected on routine X-rays following initiation of treatment, an MRI scan of both knees should be performed.
13) Medication diaries should be checked and faxed weekly during radiation therapy. After completion of XRT, medication diaries should be checked every 2 weeks and faxed every 4 weeks. Diaries should be faxed to (832)825-1198. Maintain copies of the patient diary in the research chart.

Appendix VII: Protocol Roadmap (Maintenance Therapy – Weeks 35 - 58)

Patient Initials: _____ Patient ID: _____

	Week #	Date	^Bevacizumab	^Patient's weight (kg)	*VPA dose (mg/kg/day)	*Patient's weight (kg)	Assessments
Maintenance Therapy	Week 35						**Q 2 weeks** ▪ Blood pressure ▪ Check patient diary (13) **Q 4 weeks** ▪ History, physical & neuro exam (1) ▪ Vital signs (1), performance status, height, weight, BSA, ▪ Labs: CBC, differential, platelets (2), chem 7, albumin; ALT, AST, bilirubin(2); urine protein/CR(3), Valproic acid levels (2, 6) ▪ MRI brain (10) ▪ Fax roadmap and patient diary (13) **Q 12 weeks** ▪ MRI brain (9) and spine (11) ▪ CSF cytology (5) **Q 24 weeks** ▪ X-ray of right knee (12)
	Week 36						
	Week 37						
	Week 38						
	Week 39						
	Week 40						
	Week 41						
	Week 42						
	Week 43						
	Week 44						
	Week 45						
	Week 46						
	Week 47						
	Week 48						
	Week 49						
	Week 50						
	Week 51						
	Week 52						
	Week 53						
	Week 54						
	Week 55						
	Week 56						
	Week 57						
	Week 58						

^ Bevacizumab will be administered during maintenance therapy every 2 weeks for a maximum of 24 months (including XRT). See protocol 5.3 for administration and monitoring guidelines and 5.5 for dose modifications.

* Valproic Acid (VPA) will be given daily in three divided doses. See section 5.2 for administration and monitoring guidelines and 5.4 for dose modifications.

1) For patients with newly identified punctuate hemorrhage on MRI, physical exam, neurological assessment, and performance status should be evaluated every 2 weeks at least twice to closely monitor for signs/symptoms of worsening hemorrhage.
2) Patients with grade 4 neutropenia, anemia, and/or thrombocytopenia should have CBC checked twice weekly until the cytopenia(s) improves to grade 3 or better. Patients who require a dose escalation to maintain targeted trough concentration should also have trough VPA, CBC, and liver functions measured every 3-5 days until target is reached.
3) See Appendix II for Urine/Protein Creatinine ratio.
4) For female patients of child-bearing potential
5) CSF cytology at the time of diagnosis and subsequently during protocol therapy should be performed as clinically indicated but is not required for study entry.
6) See Section 5.2.3 for monitoring valproic acid concentrations.
8) For patients with possible radiographic progression on week 10 MRI, the next MRI should be performed at week 18 (see section 8.3). If week 18 MRI shows stable disease or better compared to week 10 MRI, then patients will continue MRI every 12 weeks.

9) During maintenance therapy, MRI of brain, including GRE images, should be performed every 12 weeks until completion of protocol therapy.
10) For patients with newly identified punctate hemorrhage on MRI, the next MRI, including GRE images, should be repeated every 4 weeks at least twice to monitor possible progression of hemorrhage. In the absence of radiographic progression of hemorrhage, the frequency of MRI studies can then return to every 12 weeks.
11) For patients with high grade gliomas, an MRI of the spine should be performed at the time of study entry, but subsequent MRI of the spine should only be repeated as clinically indicated.
12) Pre-treatment tibial X-ray (AP and lateral views) of the right knee must be obtained in all patients younger than 16 years of age prior to starting maintenance therapy and be repeated every 24 weeks while receiving bevacizumab. If abnormalities of the metaphyseal plates are detected on routine X-rays following initiation of treatment, an MRI scan of both knees should be performed.
13) Medication diaries should be checked and faxed weekly during radiation therapy. After completion of XRT, medication diaries should be checked every 2 weeks and faxed every 4 weeks. Diaries should be faxed to (832)825-1198. Maintain copies of the patient diary in the research chart.

Appendix VII: Protocol Roadmap (Maintenance Therapy – Weeks 59 - 82)

Patient Initials: _____ Patient ID: _____

	Week #	Date	^Bevacizumab	^Patient's weight (kg)	*VPA dose (mg/kg/day)	*Patient's weight (kg)	Assessments
Maintenance Therapy	Week 59						**Q 2 weeks** ▪ Blood pressure ▪ Check patient diary (13) **Q 4 weeks** ▪ History, physical & neuro exam (1) ▪ Vital signs (1), performance status, height, weight, BSA, ▪ Labs: CBC, differential, platelets (2), chem 7, albumin; ALT, AST, bilirubin(2); urine protein/CR(3), Valproic acid levels (2, 6) ▪ MRI brain (10) ▪ Fax roadmap and patient diary (13) **Q 12 weeks** ▪ MRI brain (9) and spine (11) ▪ CSF cytology (5) **Q 24 weeks** ▪ X-ray of right knee (12)
	Week 60						
	Week 61						
	Week 62						
	Week 63						
	Week 64						
	Week 65						
	Week 66						
	Week 67						
	Week 68						
	Week 69						
	Week 70						
	Week 71						
	Week 72						
	Week 73						
	Week 74						
	Week 75						
	Week 76						
	Week 77						
	Week 78						
	Week 79						
	Week 80						
	Week 81						
	Week 82						

^ Bevacizumab will be administered during maintenance therapy every 2 weeks for a maximum of 24 months (including XRT). See protocol 5.3 for administration and monitoring guidelines and 5.5 for dose modifications.

* Valproic Acid (VPA) will be given daily in three divided doses. See section 5.2 for administration and monitoring guidelines and 5.4 for dose modifications.

1) For patients with newly identified punctuate hemorrhage on MRI, physical exam, neurological assessment, and performance status should be evaluated every 2 weeks at least twice to closely monitor for signs/symptoms of worsening hemorrhage.
2) Patients with grade 4 neutropenia, anemia, and/or thrombocytopenia should have CBC checked twice weekly until the cytopenia(s) improves to grade 3 or better. Patients who require a dose escalation to maintain targeted trough concentration should also have trough VPA, CBC, and liver functions measured every 3-5 days until target is reached.
3) See Appendix II for Urine/Protein Creatinine ratio.
4) For female patients of child-bearing potential
5) CSF cytology at the time of diagnosis and subsequently during protocol therapy should be performed as clinically indicated but is not required for study entry.
6) See Section 5.2.3 for monitoring valproic acid concentrations.
8) For patients with possible radiographic progression on week 10 MRI, the next MRI should be performed at week 18 (see section 8.3). If week 18 MRI shows stable disease or better compared to week 10 MRI, then patients will continue MRI every 12 weeks.

9) During maintenance therapy, MRI of brain, including GRE images, should be performed every 12 weeks until completion of protocol therapy.
10) For patients with newly identified punctate hemorrhage on MRI, the next MRI, including GRE images, should be repeated every 4 weeks at least twice to monitor possible progression of hemorrhage. In the absence of radiographic progression of hemorrhage, the frequency of MRI studies can then return to every 12 weeks.
11) For patients with high grade gliomas, an MRI of the spine should be performed at the time of study entry, but subsequent MRI of the spine should only be repeated as clinically indicated.
12) Pre-treatment tibial X-ray (AP and lateral views) of the right knee must be obtained in all patients younger than 16 years of age prior to starting maintenance therapy and be repeated every 24 weeks while receiving bevacizumab. If abnormalities of the metaphyseal plates are detected on routine X-rays following initiation of treatment, an MRI scan of both knees should be performed.
13) Medication diaries should be checked and faxed weekly during radiation therapy. After completion of XRT, medication diaries should be checked every 2 weeks and faxed every 4 weeks. Diaries should be faxed to (832)825-1198. Maintain copies of the patient diary in the research chart.

Appendix VII: Protocol Roadmap (Maintenance Therapy – Weeks 83 – 105)

Patient Initials: _____ Patient ID: _____

	Week #	Date	^Bevacizumab (mg)	*VPA dose (mg/kg/day)	Cumulative VPA dose per week (mg)	Patient's weight (kg)	Assessments
Maintenance Therapy	Week 83						**Q 2 weeks** ▪ Blood pressure ▪ Check patient diary (13) **Q 4 weeks** ▪ History, physical & neuro exam (1) ▪ Vital signs (1), performance status, height, weight, BSA, ▪ Labs: CBC, differential, platelets (2), chem 7, albumin; ALT, AST, bilirubin(2); urine protein/CR(3), Valproic acid levels (2, 6) ▪ MRI brain (10) ▪ Fax roadmap and patient diary (13) **Q 12 weeks** ▪ MRI brain (9) and spine (11) ▪ CSF cytology (5) **Q 24 weeks** ▪ X-ray of right knee (12
	Week 84						
	Week 85						
	Week 86						
	Week 87						
	Week 88						
	Week 89						
	Week 90						
	Week 91						
	Week 92						
	Week 93						
	Week 94						
	Week 95						
	Week 96						
	Week 97						
	Week 98						
	Week 99						
	Week 100						
	Week 101						
	Week 102						
	Week 103						
	Week 104						
	Week 105						

^ Bevacizumab will be administered during maintenance therapy every 2 weeks for a maximum of 24 months (including XRT). See protocol 5.3 for administration and monitoring guidelines and 5.5 for dose modifications.

* Valproic Acid (VPA) will be given daily in three divided doses. See section 5.2 for administration and monitoring guidelines and 5.4 for dose modifications.

1) For patients with newly identified punctuate hemorrhage on MRI, physical exam, neurological assessment, and performance status should be evaluated every 2 weeks at least twice to closely monitor for signs/symptoms of worsening hemorrhage.
2) Patients with grade 4 neutropenia, anemia, and/or thrombocytopenia should have CBC checked twice weekly until the cytopenia(s) improves to grade 3 or better. Patients who require a dose escalation to maintain targeted trough concentration should also have trough VPA, CBC, and liver functions measured every 3-5 days until target is reached.
3) See Appendix II for Urine/Protein Creatinine ratio.
4) For female patients of child-bearing potential
5) CSF cytology at the time of diagnosis and subsequently during protocol therapy should be performed as clinically indicated but is not required for study entry.
6) See Section 5.2.3 for monitoring valproic acid concentrations.
8) For patients with possible radiographic progression on week 10 MRI, the next MRI should be performed at week 18 (see section 8.3). If week 18 MRI shows stable disease or better compared to week 10 MRI, then patients will continue MRI every 12 weeks.
9) During maintenance therapy, MRI of brain, including GRE images, should be performed every 12 weeks until completion of protocol therapy.

10) For patients with newly identified punctate hemorrhage on MRI, the next MRI, including GRE images, should be repeated every 4 weeks at least twice to monitor possible progression of hemorrhage. In the absence of radiographic progression of hemorrhage, the frequency of MRI studies can then return to every 12 weeks.
11) For patients with high grade gliomas, an MRI of the spine should be performed at the time of study entry, but subsequent MRI of the spine should only be repeated as clinically indicated.
12) Pre-treatment tibial X-ray (AP and lateral views) of the right knee must be obtained in all patients younger than 16 years of age prior to starting maintenance therapy and be repeated every 24 weeks while receiving bevacizumab. If abnormalities of the metaphyseal plates are detected on routine X-rays following initiation of treatment, an MRI scan of both knees should be performed.
13) Medication diaries should be checked and faxed weekly during radiation therapy. After completion of XRT, medication diaries should be checked every 2 weeks and faxed every 4 weeks. Diaries should be faxed to (832)825-1198. Maintain copies of the patient diary in the research chart.

Appendix VII: Protocol Roadmap for Follow-up

Patient Initials: _____ Patient ID: _____

Date patient completed maintenance therapy: _____

Months post therapy	Date	*Vital Status		^Disease progression?		Assessments
		Alive	Deceased	No	Yes	
6 months		□	□	□	□	**Q 6 months** ▪ History, physical exam ▪ Vital signs, blood pressure ▪ Labs: CBC, differential, platelets, urine protein/creatinine ratio (see appendix II) ▪ MRI brain ▪ Fax roadmap
12 months		□	□	□	□	
18 months		□	□	□	□	
24 months		□	□	□	□	
30 months		□	□	□	□	
36 months		□	□	□	□	
42 months		□	□	□	□	
48 months		□	□	□	□	
54 months		□	□	□	□	
60 months		□	□	□	□	

* Vital Status: if deceased, date of death _____

^ Disease Progression: if yes, date of progression _____

Please fax roadmap every 6 months to (832)825-1198

APPENDIX VIII: SAMPLE INFORMED CONSENT / PARENTAL PERMISSION FOR PARTICIPATION IN RESEARCH

A Phase 2 Study of Valproic Acid and Radiation, Followed by Maintenance Valproic Acid and Bevacizumab in Children with Newly Diagnosed High-Grade Gliomas or Brainstem Gliomas

The use of "you" in this document refers to you or your child; the use of "we" in this document refers to the doctors and other study staff.

This study is a clinical trial, or a research study involving patients and a potential new treatment. Clinical trials include only patients who choose to take part. Your participation in this study is entirely voluntary. Please read the consent form carefully, and you will be given a copy of this consent if you decide to participate. You may discuss your decision with your friends and family if you would like.

You are being asked to participate in this study because you have an aggressive brain tumor called glioblastoma multiforme, anaplastic astrocytoma, gliomatosis cerebri, gliosarcoma, or brainstem glioma. These brain tumors are collectively called "malignant gliomas." This study is being conducted by the Texas-Oklahoma Pediatric Neuro-Oncology Consortium, which consists of nine pediatric cancer centers treating children with brain tumors in the state of Texas and Oklahoma.

Currently, there are few effective treatments for your type of aggressive brain tumors. Surgery and radiation can generally slow down these aggressive brain tumors, but in the majority of patients, these tumors will start growing again in 6-12 months. Adding chemotherapy drugs to surgery and radiation does not clearly improve the cure rate of children with malignant gliomas. We are conducting this study to see if the combination of valproic acid and bevacizumab (also known as Avastin™) with surgery and radiation will shrink your brain tumor more effectively and improve your chance of cure.

Valproic acid (VPA) has been given to children with seizures (epilepsy) for many decades, and it has been approved by the Food and Drug Administration (FDA) but has not been approved specifically for treating children with brain tumors. From 40 years of clinical experience using VPA in children with epilepsy, we know that children can safely take this drug for many years. Based on laboratory and animal studies, we learned that VPA may kill malignant glioma cells. We also learned that VPA may make malignant glioma cells more sensitive to radiation. In two recently completed clinical trials, VPA given as a single drug was shown to shrink malignant gliomas in some children. However, we only have limited experience in giving VPA with radiation, and we have no past clinical experience in giving VPA with bevacizumab, the second drug that you will receive on this clinical trial. Therefore, the use of VPA with radiation and bevacizumab for your brain tumor is considered investigational.

Tumors in general require extra blood vessels to support their rapid growth and high nutritional demands. A type of protein called vascular endothelial growth factor, or VEGF, has been shown to be produced at high levels in many human tumors, including malignant gliomas. Bevacizumab (Avastin™) is a specific antibody (a protein that recognizes and binds to another protein) for the VEGF protein, and in animal studies, treatment with bevacizumab has been shown to decrease VEGF levels, cut off blood vessels, and shrink many human tumors, including malignant gliomas. More than eight thousand adults have received bevacizumab, either as a single drug or in combination with chemotherapy drugs, in numerous clinical trials, and this drug has been approved by the FDA for

treatment of colon and lung cancers. Recently, clinical trials in adults with recurrent malignant gliomas (tumors that started growing after initial surgery, radiation, and chemotherapy) showed that bevacizumab can shrink these tumors in more than 25% of patients. Bevacizumab has been tested in children with recurrent solid tumors, and there is a current clinical trial testing bevacizumab and chemotherapy in children with recurrent brain tumors. Bevacizumab was recently approved by the FDA as a treatment for patients with malignant gliomas; however, the combination of VPA and bevacizumab is considered investigational.

VPA is a commercially available drug. The bevacizumab that will be used in this clinical trial is for use in research studies only. Although there may be some minor differences between the bevacizumab used for research studies only and the commercial drug Avastin™, both drugs are manufactured by a similar process and supplied by the same company (Genentech, Inc), meet similar standards for final product testing, and are expected to be similar in safety and effectiveness.

Why is this study being done?
We are testing the combination of VPA, a commonly drug for epilepsy in children, with a new drug, bevacizumab, for children who have just been diagnosed with malignant gliomas. This new combination of treatment will be given in addition to surgery and radiation, which are considered to be the standard treatment for children with malignant gliomas.

The goals of this study are:

- **To see if children with malignant gliomas treated with VPA and bevacizumab, in addition to surgery and radiation, will have a higher cure rate compared to children that received surgery, radiation, and chemotherapy in the past;**
- **To learn what kinds of side effects will occur in children with malignant gliomas when they receive VPA and bevacizumab, in addition to surgery and radiation;**
- **To learn if levels of proteins that are affected by VPA, in your brain tumor will predict your tumor's response to VPA and/or radiation;**
- **To learn if levels of proteins that allow tumor cells to repair damage to genes in brain tumors will predict tumor response to VPA and/or radiation**
- **To learn if levels of certain proteins that are affected by bevacizumab will predict the response of a tumor to bevacizumab and/or radiation**

How many people will take part in the study?
There will be about 23-34 patients participating in this study over the first 2 years. About ___ will be treated at this hospital. If the analysis at 2 years showed that this new combination is beneficial for children with malignant gliomas, we will continue to recruit more patients; if not, this clinical trial will be closed, but if you have already started this clinical trial at the time of its closure, you can still continue to receive this combination of treatment.

What will happen if I take part in this research study?
Before you begin the study …
You will need to have the following exams, tests or procedures to find out if you can be in the study. These exams, tests or procedures are part of regular cancer care and may be done even if you do not join the study. If you have had some of them recently, they may not need to be repeated. This will be up to your study doctor.

- A medical history
- Physical exam, including a neurological exam
- Vital signs (blood pressure, heart rate, temperature, weight, height, and body surface area)
- Blood tests
- Urine tests
- Pregnancy test (if you are a woman who could have children)
- MRI of brain and spine
- A lumbar puncture (a spinal tap), if your doctor thinks it is safe and required for your care
- Any other studies or tests your treating doctor thinks it is necessary to give you the best care

If you are not done growing, you will also have to have an X-ray of your knee to monitor for any side effects that may be related to bevacizumab.

Surgery
If your neurosurgeon and your treating doctor think that surgery can be safely performed, you will first have a surgery (or more than one if needed) to remove as much of your brain tumor as possible. For some of you, surgery may not be safe and will not be performed. Please discuss the safety of surgery for your brain tumor with your neurosurgeon and treating doctor.

Radiation
Once you have recovered from your surgery, you will start VPA and radiation as described in the table below. You will meet with a radiation oncologist (doctors who will be giving you radiation to treat your brain tumor) to discuss the side effects of radiation therapy. VPA will be given to you by mouth, either as capsules, syrup, or a combination of both. You will take VPA three times a day (every 8 hours), and we will ask you to keep a diary of the date and time when you take your VPA.

Treatment and Tests Required During Your Radiation Treatment			
	Monday through Friday	**Daily**	**Once a week**
Radiation	For about 6 weeks		
VPA		3 times a day, by mouth	
Exam, blood tests			Once a week; more if needed
VPA drug diary		Record with each dose	

Your radiation will last about 6 weeks. You will continue your VPA daily without interruption unless told to do so by your treating doctor.

Rest period between radiation and maintenance treatment
After you have completed your radiation, you will have a rest period of about 4 weeks before you start your maintenance treatment (combination of bevacizumab and VPA; see discussion below). Assuming that you have no side effect from VPA that require you to discontinue this drug, you will continue VPA three times daily without interruption during this rest period. If you have had side effects related to VPA and was instructed to discontinue this drug during radiation, then you will not re-start this drug until the start of your maintenance treatment (see below). Your will have an MRI at about 10 weeks after starting your radiation treatment to see if your tumor has responded to your treatment.

Treatment and Tests During the Rest Period After Radiation			
	Daily	**Every 2 weeks**	**Week 10**
VPA	3 times a day, by mouth		
Exam, blood tests		Every 2 weeks	
VPA drug diary	Record with each dose		
MRI of brain			Week 10
MRI of spine			If needed
Spinal tap			If needed

Maintenance treatment with bevacizumab and VPA
Before starting your maintenance treatment, your treating doctor will repeat most of the blood tests that you already had at the beginning, to make sure that you can still tolerate your treatment. Bevacizumab will be given to you through your vein (i.v.) in the clinic every two weeks, and VPA will be given to you by mouth three times a day, as you have been taking this drug during radiation. Even if you have experienced side effects with VPA during radiation and needed to stop this drug, we will still ask you to re-start VPA, but at a lower dose to prevent the same side effect(s) from happening again.

You will continue to receive bevacizumab and VPA, assuming that you do not experience any side effects that require you to stop either or both drugs, for a maximum period of 94 weeks. Therefore, the maximum length of your total treatment (radiation, VPA, and bevacizumab) will be two years (104 weeks). During your maintenance treatment, you will have blood tests, urine tests, and MRIs done as described in the table below. These tests are to ensure that you are not having unsafe side effects from your treatment. If you experience side effects and have to stop either bevacizumab or VPA, you have the option of remaining on this clinical trial and continuing the other drug by itself. However, you may also elect to stop participating in this clinical trial and seek other treatment options.

Treatment and Tests Required During Maintenance Therapy					
	Daily	**Every 2 weeks**	**Every 4 weeks**	**Every 12 weeks**	**Every 24 weeks**
VPA	3 times daily				
VPA drug diary	Record each dose				
Bevacizumab		i.v. every 2 weeks			
Blood pressure		Every 2 weeks			
Exam and blood tests			Every 4 weeks		
Urine tests			Every 4 weeks		
MRI of brain				Every 12 weeks	
MRI of spine				If needed	If needed
Spinal tap)					If needed
X-ray of knee					Every 24 weeks

Because VPA is given by mouth, and bevacizumab is given in your vein over 30-90 minutes, you will not have to be hospitalized to receive this treatment combination, unless you have side effects that require further treatment. You can either receive bevacizumab through a temporary catheter in your

vein, which will have to be inserted and replaced every 2 weeks, or through a permanently placed catheter called a central line (which will require a surgery). Please discuss with you treating doctor regarding the safety of placing and maintaining a central line.

Biological studies
We would like to study the levels of some proteins in your blood and brain tumor because we think that they may predict how your tumor will respond to radiation, VPA, and/or bevacizumab. In addition, by studying these proteins in your blood and brain tumor, we hope to learn more about malignant gliomas in children and design more effective treatment in the near future. Participation in these biologic studies is entirely voluntary; your refusal to participate will not prevent you from receiving the treatment already described in this consent. If you weight more than 10 kilogram, you will have two teaspoons of blood drawn before starting treatment and once more at 2 weeks after starting VPA and radiation. If you weigh less than 10 kilogram, you will have only 1 teaspoon drawn twice as described above. These amounts of blood are safe to draw from children. You can agree to have just your tumor or blood studied, or you can agree to have both studied.

Please indicate by initialing below whether you choose to participate in the biological studies.

_____/_____ Yes, I agree to participate in the biological studies.

If you answered "Yes," please choose **only** one of the following options:

_____ I only agree to have my tumor used for the biological studies.

_____ I only agree to have my blood used for the biological studies.

_____ I agree to have both my blood and tumor used for the biological studies.

_____/_____ No, I do not agree to participate in the biological studies.

How long will I be in the study?
You may be in the study for up to 24 months if you are responding to therapy and not having side effects that are dangerous for you. Your doctor may decide to take you off study if any of the following occur:

- The side effects of VPA and/or bevacizumab are too harmful for you
- You need a treatment that is not allowed on this study
- Your tumor has grown back or become larger despite receiving VPA and bevacizumab
- You are not able to follow study-related treatment instructions
- New information becomes available
- The study is not in your best interest
- The study is stopped because there is no benefit for your type of brain tumor

After you are taken off treatment or after you have completed your treatment, your treating doctor will ask you to return for follow up exams and tests to ensure that you are not having dangerous side effects. Please continue to follow these instructions to ensure your safety.

Can I stop being in the study?
Yes. You can decide to stop at any time. Tell the study doctor if you are thinking about stopping or decide to stop. He or she will tell you how to stop safely.

It is important to tell the study doctor if you are thinking about stopping so any risks from VPA and/or bevacizumab can be evaluated by your doctor. Another reason to tell your doctor that you are thinking about stopping is to discuss what follow-up care and testing could be most helpful for you.

The study doctor may stop you from taking part in this study at any time if he/she believes it is in your best interest; if you do not follow the study rules; or if the study is stopped.

What side effects or risks can I expect from being in the study?

Side Effects of Valproic Acid
The side effects that we think valproic acid might have are listed below. There might be other side effects that we do not know about yet because we have only limited experience in using this drug to treat brain tumors in children. These side effects could be dangerous or life threatening. We will be checking you closely to see if any side effects are happening. Side effects of drugs like valproic acid usually get better if the treatment is stopped, but in some cases they can be serious and/or irreversible. There is the risk of death. If you do have side effects, we may recommend medicine or treatments to try to control them and make you comfortable. You should talk to your study doctor as soon as possible about any side effects that you have while taking part in the study.

Risks and side effects related to the valproic acid include the following:

Likely:
- **nausea**
- **vomiting**
- **abdominal pain**
- **diarrhea**
- **headache**
- **sleepiness**
- **tremor**
- **loss of strength and energy**
- **dizziness**
- **hair loss**
- **rash**
- **dry skin**
- **itching of the skin**
- **numbness and tingling of your hands and/or feet**
- **short-term weakness of your hands and/or feet**

Less Likely:
- **loss of appetite**

- **heartburns**
- **flu-like syndromes**
- **double vision**
- **blurred vision**
- **unsteadiness**
- **unsteady eye movement**
- **sleeplessness**
- **loss of memory**
- **moodiness**
- **anxiety or nervousness**
- **ringing in the ear**
- **weight gain or loss**
- **abnormal thyroid function**
- **low platelet count, which may cause easy bruising or bleeding**
- **abnormal blood clotting which can lead to excessive bleeding during and after surgery**

Rare but can be serious:

- **pneumonia**
- **sores in the mouth or on the tongue**
- **hyperactivity**
- **confusion**
- **coma**
- **severe reactions involving skin, mouth, and eyes, including blisters**
- **redness, swelling, and/or peeling of your skin, resulting in pain and infection**
- **eye pain and irritation**
- **low white and/or red blood cell count**
- **abnormal effects on unborn babies (see "Risks of Pregnancy" below)**
- **severe to life-threatening inflammation of the pancreas and liver**
- **hearing loss**
- **abnormal sodium level in the blood**
- **fast and/or irregular heart beats, high blood pressure**
- **peeing frequently, vaginal yeast infection**
- **inflammation of the kidney**
- **irregularities in your periods, including complete stoppage of your periods**
- **gum bleeding or swelling, infection around your teeth**
- **abnormal change in the bone marrow leading to chronic transfusions, and possibly leading to a pre-leukemia condition called myelodysplastic syndrome.**
- **abnormal clotting tests and possibly increased risk of bleeding**

Risks of Pregnancy: The drug on this study may affect an unborn baby. You should not become pregnant or father a baby while on this study. You should not nurse (breast feed) a baby while on this study. Ask about counseling and more information about preventing pregnancy.

Possible Drug Interactions Between Valproic Acid and Other Medications
Valproic acid has interactions with multiple anti-seizure medications. Valproic acid also interacts with several other medications, including acyclovir, multiple antibiotics (azithromycin, erythromycin, imipenem, meropenem, etc.), aspirin, chlorpromazine, cholestyramine, isoniazid, rifampin, amitriptyline, nortriptyline, clonazepam, diazepam, nimodipine, tricyclic anti-depressants, tolbutamide, warfarin, and zidovudine. While you are allowed to take valproic acid and any of these medications together, you should notify your treating doctor immediately if another doctor starts you on one of these medications, because you will have to be closely monitored for valproic acid levels and the side effects of these medications.

Side Effects of Bevacizumab
The side effects that we think bevacizumab might have are listed below. There might be other side effects that we do not know about yet because we have only limited experience in using bevacizumab to treat brain tumors in children. These side effects could be dangerous or life threatening. We will be checking you closely to see if any side effects are happening. Side effects of drugs like bevacizumab usually get better if the treatment is stopped, but in some cases they can be serious and/or irreversible. There is the risk of death. If you do have side effects, we may recommend medicine or treatments to try to control them and make you comfortable. You should talk to your study doctor as soon as possible about any side effects that you have while taking part in the study.

Likely side effects:
- **Nose bleeds**
- **High blood pressure – in most patients, blood pressure can be controlled with routine medications. Rarely, uncontrolled high blood pressure may lead to damage to the brain and other vital organ functions.**
- **Fatigue**
- **Skin reactions (itching, rash, flaking of the skin, or hives)**
- **Headache**
- **Soreness in mouth or throat**
- **Stuffy or runny nose, sneezing, watery eyes, abnormal reactions in the nose or sinuses**
- **Nausea or vomiting, loss of appetite**
- **Diarrhea**
- **Heartburn**
- **Abnormal blood chemistry or electrolytes**
- **Generalized pain or pain at the tumor site**
- **Voice changes, hoarseness, laryngitis**
- **Cough and/or shortness of breath**
- **Dizziness**

Less likely side effects
- **Mild to moderate bleeding in the tumor, stomach, intestine, genitourinary tract, or other parts of the body**

- **Blood clots in the veins of the leg, lungs, or other parts of the body. These clots can be life-threatening.**
- **Clots in the arteries, including stroke or heart attack. These conditions can be life-threatening or fatal.**
- **Leakage of protein in the urine, which can rarely lead to damage to the kidney or kidney failure.**
- **Reaction to bevacizumab – these reactions may include skin reactions (itching, rash, swelling, flaking of skin, and hives), respiratory symptoms (shortness of breath, cough, throat tightness, wheezing), cardiac symptoms (low blood pressure, dizziness), or others (chill, shivering)**
- **Constipation**
- **Decrease in white cell count (neutropenia) which may increase your risk of an infection**
- **Decrease in red cell count, which may require a blood transfusion**

Rare but serious side effects

- **Reversible posterior leukoencephalopathy syndrome (RPLS) or similar leukoencephalopathy syndrome: RPLS is a medical condition related to leakiness of blood vessels in the brain and can cause confusion, blindness or vision changes, seizure, and other symptoms, as well as changes on your MRI. This condition is usually reversible, but in rare cases, it is potentially life-threatening and may have long-term side effects on brain functions.**
- **Serious or fatal bleeding into the tumor, brain, gut, lungs, or other parts of the body**
- **Bowel perforation and bowel anastomotic dehiscence. Bowel perforation occurs when an opening exists in the bowel wall allowing bowel contents to spill into the abdomen. Bowel anastomotic dehiscence is a breakdown in the surgical connection between two pieces of bowel. These events are rare in patients who have not had prior bowel surgeries, but they can lead to serious infections and will require surgery to repair the breaks.**
- **Fistula formation: defect in the walls of luminal organs such as the upper airway, lungs, esophagus, rectum, or vagina. Fistula formation may lead to life-threatening complications, including serious infections, bleeding, or dysfunctions of organs.**
- **Heart problems (including irregular heartbeats, fluid collections around the heart, heart attack, or heart failure).**
- **Stroke**
- **Fluid collection within tissues of the lungs**
- **Delayed or poor wound healing**
- **Severe allergic reactions that result in difficulty breathing, a severe drop in blood pressure, and possible death**
- **Reversible changes in the liver functions**
- **Serious and sometimes fatal infection when your white cell count is very low (severe neutropenia)**
- **Sudden death of uncertain relationship to bevacizumab**

Growth and Development Risks Associated with Bevacizumab

Animal studies of drugs that decrease blood vessels in tumor, such as bevacizumab, showed decreases in ovarian function and abnormal bone growth. These and other effects of bevacizumab may

potentially impair growth and development and decrease your chance of conceiving a child in the future.

Abnormal changes in the bones after bevacizumab treatment have been observed in young children with developing bones. This side effect appeared to be reversible after the treatment was stopped, but its exact effect on bone development has not been fully understood with long-term use of this drug.

Reproductive Risks Associated with Bevacizumab
If you are able to become pregnant, a blood test will be performed before the study to ensure that you are not pregnant. Because bevacizumab can potentially affect an unborn baby and infants, you should not become pregnant or father a baby or breast feed while you are on this study. Also, bevacizumab remains in your body for weeks to months, and therefore you should continue to use adequate contraceptive measures and avoid nursing a baby for at least 6 months after your last dose of bevacizumab.

Possible Side Effects of Radiation Therapy to the Brain
Short-term side effects
- **Hair loss, both temporary and permanent**
- **Irritation or redness of the skin with possible peeling of the skin at the sites of radiation**
- **Areas of the ear canal exposed to radiation may develop inflammation and irritation weeks to months after treatment, causing plugging of ears and poor balance**
- **Nausea, vomiting, and poor appetite**
- **Weight loss from poor appetite**
- **Fatigue**
- **Short-term memory problems (difficulty remembering things, difficulty concentrating)**
- **Sleeping too much**

Long-term side effects
- **Memory difficulties (difficulty remembering things, recalling things, performing complicated tasks, etc.)**
- **Learning difficulties (problems with learning new tasks or skills, problems with academic performance in school, etc.)**
- **Hearing loss**
- **Changes in hormone function and levels, sometimes requiring hormone replacement**
- **Growth failure, sometime requiring growth hormone**
- **Vision problems**

You should discuss the potential side effects of radiation therapy in details with your radiation oncologist.

Possible Side Effects of Surgery to Remove Your Brain Tumor
Surgery to remove part or all of your brain tumor will only be performed if your neurosurgeon and your treating physician believe that such a surgery can be safely performed and its benefit outweighs its potential risk. Please discuss the benefit and risk of the surgery for your brain tumor with your neurosurgeon and treating physician.

Risks of blood drawing or placing an intravenous catheter for blood drawing
Risks associated with drawing blood are slight, but some risks include: pain, excessive bleeding, fainting or feeling lightheaded, bruising, infection (a slight risk any time the skin is broken), and multiple punctures to locate veins.

Lumbar puncture risks
A lumbar puncture to see if your brain tumor has spread to the spines will only be performed if your treating physician thinks that it is necessary for the best care of your brain tumor. A lumbar puncture has a small risk of infection, bleeding, nerve damage, or headache. Leakage of spinal fluid into the tissue can cause a severe headache that lasts for days to weeks. Patients will be given medications to numb the pain and blur the memory before the test since it is painful. The pain is usually brief but occasionally may linger. Sometimes this procedure is done while the patient is under general anesthesia.

We will tell you if we learn any new information that may affect your health, welfare, or decision to stay in this study.

If you have more questions about the risks and side effects of this study, please discuss them in details with your treating doctor before consenting for the study.

Are there benefits to taking part in the study?
The potential benefit of this new treatment with valproic acid and bevacizumab is that it may shrink your tumor more effectively than with surgery and radiation alone and therefore may improve your chance of cure. However, we do not know if you will benefit from taking part in this study. Information learned from this study may help future children with malignant gliomas.

What other choices do I have if I do not take part in this study?
- **Treatment with surgery and radiation, with or without chemotherapy drugs that have been previously tried with your type of brain tumor.**
- **Other types of investigational trial (if available).**
- **No further treatment, and comfort care only.**

Please discuss these options with your treating doctor as well as other trusted persons or family members.

Will my medical information be kept private?
We will do our best to make sure that the personal information in your medical record will be kept private. However, we cannot guarantee total privacy. Your personal information may be revealed if required by law. If information from this study is published or presented at scientific meetings, your name and other personal information will not be used.

Your medical records are available to those caring for you at this hospital. Other people or groups who may see or copy your medical record because you are participating in this study include:
- **Physicians from other institutions in the Texas-Oklahoma Pediatric Neuro-Oncology Consortium**

- **The FDA, the National Cancer Institute (NCI), or other governmental agencies involved in keeping research studies safe for children**
- **The Institutional Review Board of your hospital**
- **Representatives from Genentech, Inc. (the company that supplies bevacizumab)**

Otherwise your name will not be released without your written permission, unless required by law. If results of this study are published, your identity will remain confidential.

What are the costs of taking part in this study?
Genentech, Inc will provide bevacizumab at no cost to you and your treating doctor. You will not have to pay for the tumor biology studies if you choose to participate. Otherwise, you and/or your insurance company will be responsible for the cost of surgery, radiation, valproic acid, and all the office visits, blood tests, urine tests, MRI studies, and any other tests necessary to monitor your tumor, ensure your safety, or treat your side effects while you are on this study. You will not be paid any money for participating in this clinical trial. Please ask to speak to a financial counselor if you have any questions about the costs of being in this study.

For more information on clinical trials and insurance coverage, you can visit the National Cancer Institute's Web site at *http://cancer.gov/clinicaltrials/understanding/insurance-coverage* . You can print a copy of the "Clinical Trials and Insurance Coverage" information from this Web site.

Another way to get the information is to call 1-800-4-CANCER (1-800-422-6237) and ask them to send you a free copy.

What happens if I am injured because I took part in this study?
It is important that you tell your study doctor, _____ *[investigator's name(s)],* if you feel that you have been injured because of taking part in this study. You can tell the doctor in person or call him/her at _____ *[telephone number]*.

You will get medical treatment if you are injured as a result of taking part in this study. You and/or your health plan will be charged for this treatment. The study will not pay for medical treatment.

What are my rights if I take part in this study?

Taking part in this study is your choice. You may choose either to take part or not to take part in the study. If you decide to take part in this study, you may leave the study at any time. No matter what decision you make, there will be no penalty to you, and you will not lose any of your regular benefits. Leaving the study will not affect your medical care. You can still get your medical care from our institution.

We will tell you about new information or changes in the study that may affect your health or your willingness to continue in the study.

In the case of injury resulting from this study, you do not lose any of your legal rights to seek payment by signing this form.

Whether you participate or not, you will continue to get the best medical care this hospital can provide.

Who can answer my questions about the study?
You can talk to your study doctor about any questions or concerns you have about this study. Contact your study doctor _____ *[name(s)]* at _____ *[telephone number]*. You can also contact Dr. Jack Su, the study chair of this clinical trial, by phone (832-822-4306) or by e-mail (jmsu@txccc.org).

For questions about your rights while taking part in this study, call the _____ *[name of center]* Institutional Review Board (a group of people who review the research to protect your rights) at _____ *(telephone number). [Note to Local Investigator: Contact information for patient representatives or other individuals in a local institution who are not on the IRB or research team but take calls regarding clinical trial questions can be listed here.]*

Where can I get more information?
You may call the National Cancer Institute's Cancer Information Service at:

- 1-800-4-CANCER (1-800-422-6237) or TTY: 1-800-332-8615

You may also visit the NCI Web site at http://cancer.gov/

- For NCI's clinical trials information, go to: http://cancer.gov/clinicaltrials/
- For NCI's general information about cancer, go to http://cancer.gov/cancerinfo/

You will get a copy of this form. You will be given a copy of the protocol (full study plan) upon request. If you want more information about this study, ask your study doctor.

A description of this clinical trial will be available on http://www.ClinicalTrials.gov, as required by U.S. Law. This Web site will not include information that can identify you. At most, the Web site will include a summary of the results. You can search this Web site at any time.

SIGNATURES

I have been given a copy of all 13 pages of this form.
I have read it or it has been read to me.
I understand the information and have had my questions answered. I agree to take part in this study.

PATIENT NAME: _____
(Print Name) (Date)

PARENT (OR GUARDIAN) _____
(Signature) (Date)

PHYSICIAN OR INVESTIGATOR _____
(Signature) (Date)

WITNESS _____
(Signature) (Date)