SUMMARY OF CHANGES

For Protocol Amendment #7 (v.6.0) to: A Phase I/ II Study of ABT-888, an Oral Poly(ADP-ribose) Polymerase Inhibitor, and Concurrent Radiation Therapy, Followed by ABT-888 and Temozolomide, in Children with Newly Diagnosed Diffuse Pontine Gliomas (DIPG)

NCI Protocol #: PBTC-033
Local Protocol #: PBTC-033

NCI Version Date: February 4, 2014
Protocol Date: February 4, 2014
Updated Date: February 4, 2014

Version 5.1: Administrative Changes by Principal Investigator in response to recommendations following review of v5.0 dated 12/15/13:

#	Section	Page	Change
1.	7.3.1	47	**Please correct the paragraph below :** In the rare occurrence when Internet connectivity is lost, a 24-hour notification is to be made to CTEP by telephone at 301-897-7497. Once Internet connectivity is restored, the 24-hour notification phoned in **or entered** electronically into AdEERS by the original submitter at the site. Also, if internet connectivity is not re-established within 24 hours, notify the following by telephone and or email. **Response – revised as requested:** Expedited AE reporting for this study must use CTEP-AERS (Adverse Event Expedited Reporting System), accessed via the CTEP Web site (http://ctep.cancer.gov). The reporting procedures to be followed are presented in the "NCI Guidelines for Investigators: Adverse Event Reporting Requirements for DCTD (CTEP and CIP) and DCP INDs and IDEs" which can be downloaded from the CTEP Web site (http://ctep.cancer.gov). These requirements are briefly outlined in the tables below (section 7.3.3). In the rare occurrence when Internet connectivity is lost, a 24-hour notification is to be made to CTEP by telephone at 301-897-7497. Once Internet connectivity is restored, the 24-hour notification phoned in must be entered electronically into CTEP-AERS by the original submitter at the site. Also, if internet connectivity is not re-established within 24 hours, notify the following by telephone and or email.
2.	8.1	50	**Please note that the protocol includes the following patient care instructions:** [redacted]

			[redacted]

Version 6.0 Changes by Principal Investigator

#	Section	Page	Change
1.			Adverse Event Expedited Reporting System (CTEP-AERS)" has been changed to "CTEP Adverse Event Reporting System (CTEP-AERS)" throughout the protocol.
2.	3.1.6, 6 & 10	26 42 & 63	**Request in CTEP RRA from Dr. Nita Seibel dated 1/16/14:** Specific Protocol Revisions to Address Risk Mitigation Plan: **Response:** Our eligibility requirements, frequency of liver function testing and dose modifications meet the recommendations in the RRA dated 1/16/14
3.	7.1.2	46	Table 14 revised to include 'Hepatic failure' in response to CTEP RRA from Dr. Nita Seibel dated 1/16/14.

Table 14

Known Toxicities of Temozolomide			
	Likely (≥20%)	Less Likely (≤20%)	Rare but Serious (<5%)
Immediate: within 1-2 days of receiving drug	Anorexia Constipation Nausea Diarrhea	~~Anorexia~~, Abdominal pain, ~~Constipation, Diarrhea,~~ Headache, Itching, ~~Nausea,~~ Rash, Urinary frequency and/or infection, Vomiting, Dizziness, Anxiety, Confusion	Ataxia, Convulsions, Dysphagia, Hemiparesis, Thromboembolism
Prompt: Within 2-3 weeks, prior to next course	Myelosuppression	Lethargy, Mucositis, Peripheral edema; Fever (associated with low neutrophil count), Weight gain, Back pain, Breast pain, peripheral neuropathy, Upper respiratory infection, cough, sore throat, Myalgia, Amnesia, Depression, Visual changes, Insomnia	Myelosuppression for prolonged period with increased risk of infection or death; Hepatic failure
Delayed: Anytime later during therapy, excluding the above conditions		Alopecia~~, Hepatotoxicity~~	Hepatotoxicity
Late: Anytime after completion of therapy			Secondary tumors or cancers

NCI Protocol #: *PBTC-033*

Local Protocol #: *PBTC-033*

TITLE: PBTC-033 A Phase I/ II Study of ABT-888, an Oral Poly(ADP-ribose) Polymerase Inhibitor, and Concurrent Radiation Therapy, Followed by ABT-888 and Temozolomide, in Children with Newly Diagnosed Diffuse Pontine Gliomas (DIPG)

Coordinating Center: Operations and Biostatistics Center (OBC) for the Pediatric Brain Tumor Consortium (PBTC), St. Jude Children's Research Hospital

Principal Investigator:
Patricia A. Baxter, M.D.
Pediatric Hematology-Oncology
Texas Children's Cancer Center
Baylor College of Medicine
1102 Bates Street Suite 1030.17
Houston Texas 77030
Tel: 832-824-4681
Fax: 832-825-4038
Email: pabaxter@txch.org

Co-Chair
Jack Su, M.D., M.S.
Pediatric Hematology-Oncology
Texas Children's Cancer Center
Baylor College of Medicine
6701 Fannin Street, CC1410
Houston, Texas 77030
Tel: 832-822-4306
Fax: 832-825-1503
E-mail: jmsu@txch.org

Co-Investigators
Susan Blaney, MD, TXCCC
smblaney@txch.org
Patrick Thompson, MD
pathomps@txch.org
Adekunle Adesina MD, PhD TXCCC*
amadesin@txch.org

Radiation Oncology Coordinator
*Arnold Paulino, MD**
Methodist Hospital
Houston, TX
apaulino@tmhs.org

Biostatistician
Arzu Onar, PhD
Department of Biostatistics
St. Jude Children's Research Hospital
262 Danny Thomas Place
Memphis, TN 38105
Tel: 901-595-5499
Fax 901-595-8843
*Email:*arzu.onar@stjude.org

Biostatistician
Catherine Billups
Department of Biostatistics
St. Jude Children's Research Hospital
262 Danny Thomas Place
Memphis, TN 38105
Tel: 901-595-3709
Fax 901-595-4585
Email: catherine.billups@stjude.org

PBTC Protocol Coordinator
Stacye Richardson, MSHS, BSMT, CCRP
Department of Biostatistics
St. Jude Children's Research Hospital
262 Danny Thomas Place
Memphis, TN 38105
Telephone: 901-595-3783
Fax: 901-595-4585
Email: stacye.richardson@stjude.org

Pharmacokinetics Laboratory

[redacted]

Biology Correlative Study Laboratory

[redacted]

Biology Correlative Study Laboratory
*Xiao-Nan Li, MD, PhD**
Texas Children's Cancer Center
1102 Bates, Rm 1030
Houston, TX 77030
Tel: 832-824-4580
Fax : 832-825-1503
Email: xxli@txch.org

CRA/Nursing Committee Reps
Susan Burlingame, TXCCC
Email: sxburlin@txch.org

Correlative Study Laboratory – Urine Biomarkers
*Edward R. Smith, MD**
Director, Pediatric Cerebrovascular Surgery
Department of Neurosurgery
Children's Hospital Boston / Harvard Medical School
300 Longwood Avenue
Boston, MA 02115
Tel: 617-355-8414
Fax: 617-730-0906
E-mail:
edward.smith@childrens.harvard.edu

PBTC Neuroimaging Center
*Tina Young Poussaint, M.D.**
Director, PBTC Neuroimaging Center
Children's Hospital of Boston
Department of Radiology
300 Longwood Avenue
Boston, MA 02115
Tel: 617- 355-6450
Fax: 617-730-0573
Email:
tina.poussaint@childrens.harvard.edu

Neuroradiology Co-Investigators
*Meng Law, MD and Mark Shiroishi, M.D.**
Division of Neuroradiology
Department of Radiology
Keck School of Medicine
University of Southern California
1500 San Pablo St
Los Angeles, CA 90033
*Tel:*323-442-7483
*Fax:*323-226-4059
*Email:*meng.law@usc.edu
Email: mshiroishi@gmail.com

NCI Supplied Agent: ABT-888 (Veliparib)

IND Sponsor: CTEP/NCI/NIH

Commercial Agent: Temozolomide (Temodar™)/NSC# 362856
Temozolomide is available from commercial sources by the treating hospital pharmacy.

Protocol Type / Version # / Version Date: Amendment/Version 6.0/ February 4, 2014

Pediatric Brain Tumor Consortium Institutions and Principal Investigators

James M. Boyett, Ph.D.*
Executive Director of OBC for the PBTC
St. Jude Children's Research Hospital
Department of Biostatistics
262 Danny Thomas Place
Memphis, TN 38105
TEL: (901) 595-3370
FAX: (901) 595-8843
EMAIL: james.boyett@stjude.org

Murali Chintagumpala, M.D.
Baylor College of Medicine
Texas Children's Cancer Center
Dept. of Pediatrics
6621 Fanin St. C1510
Houston, Texas 77030
TEL: (832) 822-4266
FAX: (832) 824-4202
EMAIL: mxchinta@txch.org

Ian Pollack, M.D.
Children's Hospital of Pittsburgh of UPMC
FP, 4th Floor – Neurosurgery Department
Children's Hospital Drive
45th and Penn Avenue
Pittsburgh, Pennsylvania 15201
TEL: (412) 692-5881
FAX: (412) 692-5921
EMAIL: Pollaci@chp.edu

Roger Packer, M.D.
Children's National Medical Center
Dept. of Neurology
111 Michigan Avenue, NW
Washington, DC 20010
TEL: (202) 884-2120
FAX: (202) 884-5226
EMAIL: rpacker@cnmc.org

Paul Graham Fisher, M.D.
Stanford University and Lucile Packard Children's Hospital
Neurology and Pediatrics, Chief, Division of Child Neurology
750 Welch Road, Suite 317, Palo Alto, CA 94304-1510
TEL: (650) 721-5889
FAX: (650) 723-7299
EMAIL: pfisher@stanford.edu

Ira Dunkel, M.D.
Memorial Sloan Kettering Cancer Center
Department of Pediatrics
1275 York Avenue , New York, NY 10065
TEL: 212-639-2153
FAX: 212-717-3239
EMAIL: dunkeli@mskcc.org

Larry Kun, M.D.
St. Jude Children's Research Hospital
Dept. of Radiation Oncology
262 Danny Thomas Place
Memphis, Tennessee 38105-2794
TEL: (901) 595-3565
FAX: (901) 595-3113
EMAIL:larry.kun@stjude.org

Sridharan Gururangan, M.D.
Duke University Medical Center
The Preston Robert Tisch Brain Tumor Center
Duke University Medical Center
047 Baker House, Trent Drive
Durham, North Carolina 27710
TEL: (919) 684-3506
FAX: (919) 668-2485
EMAIL: gurur002@mc.duke.edu

Kathy Warren, M.D.
NIH/NCI/NOB
National Cancer Institute
Pediatric Oncology Branch
Bldg.10 CRC, Room 1-5750
9000 Rockville Pike
Bethesda, MD 20892
TEL: (301) 435- 4683
FAX: (301) 480-2308
EMAIL: warrenk@mail.nih.gov

Stewart Goldman, M.D.
Children's Memorial Hospital
2300 Children's Plaza
Chicago, IL 60614-3394
TEL: (773) 880-4562
FAX: (773) 880-3223
EMAIL: sgoldman@northwestern.edu

Maryam Fouladi, M.D.
Cincinnati Children's Hospital Medical Center
Dept. Of Hematology/Oncology
MLC 7015
3333 Burnet Ave,
Cincinnati, OH 45229
TEL: (513) 803 0721
FAX: (513) 636-3549
EMAIL: maryam.fouladi@cchmc.org

Girish Dhall, MD
Children's Hospital Los Angeles
Children's Center for Cancer and Blood Diseases
Neuro-Oncology Program,
Mail Stop #54
4650 Sunset Blvd; Los Angeles, CA 90026
TEL: 323-361-4629
FAX: 323-361-8165
EMAIL: gdhall@chla.usc.edu

**** NOT RESPONSIBLE FOR PATIENT CARE***

PROTOCOL ABSTRACT AND SCHEMA

This is a phase I/II study to determine: 1) the maximum tolerated dose (MTD) or recommended phase II dose of ABT-888 in combination with radiation therapy, and 2) the efficacy of administering ABT-888 concurrently with radiation therapy, followed by maintenance therapy with ABT-888 and temozolomide (TMZ), in children with newly diagnosed diffuse intrinsic pontine glioma (DIPG).

This study consists of two parts: the phase 1, dose-finding component of the trial to estimate the MTD or recommended phase II dose of ABT-888 in combination with radiation therapy, and the phase 2 component of the study to evaluate the efficacy of ABT-888 and radiation therapy, followed by maintenance therapy with ABT-888 and TMZ, in children with newly diagnosed DIPG. Upon completion of ABT-888 and radiation therapy, patients enrolled in the Phase I study will continue with maintenance therapy with ABT-888 at 25 mg/m^2 bid and TMZ at 135 mg/m^2/day for 5 days every 28 days, the recommended phase II doses determined from PBTC-027. After Phase I is completed, all patients will receive radiation and ABT-888, at the MTD/recommended phase II dose determined from the Phase I study, followed by maintenance therapy with ABT-888 at 25 mg/m^2 bid and TMZ at 135 mg/m^2/day for 5 days every 28 days, the recommended phase II doses determined from PBTC27.

Since PBTC-027 enrolled patients who commonly received multiple prior chemotherapy treatments, and patients for PBTC-033 will be newly diagnosed, chemotherapy-naïve, and presumably have intact bone marrow reserve, we hypothesize that children for this clinical trial may tolerate higher combination doses of ABT-888 and TMZ compared to the children studied on PBTC-027. Therefore, during the maintenance therapy phase of both the Phase I and Phase II studies, intra-patient dose escalation of TMZ will be studied. Each patient will start maintenance therapy with ABT-888 at 25 mg/m^2 bid and TMZ at 135 mg/m^2/day for 5 days every 28 days. The TMZ dose will be escalated to 175 mg/m^2/day and then to 200 mg/m^2/day for 5 days every 28 days if no minimal toxicity is observed after each course.

The primary endpoints for the Phase I study will be toxicity and safety monitoring of ABT-888 in combination with radiation therapy and will include DLTs and toxic death. Dose-modifying toxicities for maintenance therapy will also be monitored to guide intra-patient dose escalation of TMZ and to terminate intra-patient dose escalation if excessive toxicity is observed (section 5.1.3). The primary endpoint for the Phase II study will be the evaluation of the treatment efficacy as measured by 1-year overall survival (OS), with 1-year progression-free survival (PFS) and best tumor responses observed prior to tumor progression as the secondary measures of treatment efficacy.

PARP activity, non-homologous end-joining (NHEJ) activity, and γ–H2AX level in peripheral blood monocytes (PBMCs) will be quantified pre-treatment, 2 weeks after starting ABT-888 and irradiation, during the last week of ABT-888 and irradiation, and during the first course of maintenance therapy, as surrogate measures of intra-tumoral PARP inhibition and unrepaired double-stranded DNA breaks (DSB). In atypical pontine gliomas that are biopsied, PARP activity and DNA repair protein status will be studied either in frozen tumor material or FFPE samples as described in section 9.1.3. These molecular data will be correlated retrospectively

with efficacy measures outlined above. Pharmacokinetic studies of ABT-888 on day 1 and day 4 of ABT-888 and radiation treatment will also be performed. These PK studies will be required for Phase I and optional for Phase II of the study.

To differentiate pseudoprogression from true early progressive disease, quantitative MR measures of relative cerebral blood volume (rCBV), permeability (Ktrans, vp, and ve values), and apparent diffusion coefficient (ADC) will be obtained at diagnosis and during the first six months after starting protocol therapy and correlated with disease outcome, including whether such metrics differentiate patients with pseudoprogression from those with true early progressive disease.

Urine biomarkers will be employed to study the feasibility of a novel, non-invasive method to detect the presence, progression and response to therapy of pediatric brain tumors.

Schema:
Phase I Study
ABT-888 will be given twice daily on Monday through Friday, for 6-7 weeks, during daily radiation therapy (see table below). The first dose of ABT-888 should ideally be given 60-120 minutes prior to timing of radiation treatment. For patients who require sedation for radiation treatment and must remain NPO in the morning, ABT-888 should be given nightly prior to sleep, with the day time dose given shortly after the child awakens from sedation, adhering as closely to an every 12 hour schedule as possible.

Schema – Radiation Phase of Therapy							
Week	1	2	3	4	5	6	Week 7-10
ABT-888, BID	Twice daily, M-F, for 6-7 weeks						Rest/ Evaluation
Radiation Therapy	Daily, M-F for 6-7 weeks						

Dose Level 1 of ABT-888 during radiation treatment will be approximately 80% of the dose that has been safely tolerated in adults (100 mg BID). It is also expected that the 150 mg bid dose level in adults will be declared tolerable shortly. Dose escalation/de-escalation will be done using a standard phase I, 3 + 3 design in increments of approximately 30% (see the table below). If the adult dose of 150 mg bid with radiation has been declared tolerable by the time this trial begins patient accrual, and the starting dose level of 50 mg/m2 bid is also tolerated in children, we propose to proceed from dose level 1 directly to dose level 3 (85 mg/m2 bid, equivalent to the adult dose of 150 mg bid). If dose level 3 is not tolerated, then we will de-escalate to dose level 2. If dose level 3 is tolerated, we will study dose level 4 only if supported by adult clinical data and PBMC PARP inhibition data from our ongoing trial.*

ABT-888 Dose Level During Radiation, for Phase I	
Dose Level	**ABT-888 dose (mg/m^2/dose BID), M-F**
0	35 mg/m^2/dose BID
1 (starting dose level)	50 mg/m^2/dose BID
2	65 mg/m^2/dose BID
3	85 mg/m^2/dose BID
4 (pending supporting data)*	110 mg/m^2/dose BID

Upon completing radiation treatment and ABT-888, all patients from Phase I of the study will continue with maintenance therapy starting week 11, as outlined below.

Schema- Maintenance Phase of Therapy						
Each Course	**May receive a maximum of 10 courses**					
Day	1	2	3	4	5	6-28
ABT-888, BID	x,x	x,x	x,x	x,x	x,x	Rest/Evaluation
Temozolomide, once daily	x	x	x	x	x	Rest/Evaluation

Phase II Study

Phase II of the study will begin when the MTD/recommended phase II dose of ABT-888 in combination with radiation therapy has been determined from the Phase I study. Maintenance therapy will consist of ABT-888 and TMZ beginning at week 11 (3-4 weeks after the completion of radiation and ABT-888). Starting doses of maintenance therapy will be 25 mg/m^2 bid of ABT-888 and 135 mg/m^2/day of TMZ, for 5 days every 28 days, which are the recommended phase II doses from PBTC-027. Since this dose determination was based on children with refractory brain tumors, many of whom were heavily pre-treated, it is possible that children with newly diagnosed DIPG, who are chemotherapy naïve at entry, will tolerate higher doses of ABT-888 and TMZ during maintenance. In an effort to maximize the TMZ dose (and potentially the efficacy of the combination treatment) that can be administered with ABT-888 for each patient, **intra-patient dose escalation of TMZ will be studied only for patients with minimal hematologic toxicities in each course, defined as ≤ Grade 1 thrombocytopenia and ≤ Grade 2 neutropenia, and minimal non-hematologic toxicities not meeting the definition of dose-modifying toxicities in section 5.1.3.1,** after their first course of maintenance therapy at Dose Level 1. These patients' TMZ dose will be escalated for Course 2 to 175 mg/m^2/day (Dose Level 2), and subsequently (Course 3) to 200 mg/m^2/day of TMZ (Dose Level 3) if the toxicities observed after 1 course of protocol therapy at each dose level meet the above criteria.

Intra-Patient Dose Escalation during Maintenance Therapy (Days 1-5 per 28 day cycle)		
Dose Level	**ABT-888**	**TMZ Dose**
0	20 mg/m^2 BID	135 mg/m^2/day
1 (Starting Dose)	25 mg/m^2 BID	135 mg/m^2/day
2	25 mg/m^2 BID	175 mg/m^2/day
3	25 mg/m^2 BID	200 mg/m^2/day

TABLE OF CONTENTS

1. OBJECTIVES

1.1 Phase I Primary Objectives

1. To identify the maximum tolerated dose or recommended Phase II dose of ABT-888 which can be safely administered concurrently with radiation therapy, followed by maintenance therapy with ABT-888 and TMZ, in patients with newly diagnosed DIPG.
2. To study the plasma pharmacokinetics (PK) of ABT-888 during ABT-888 and radiation therapy.
3. To study the feasibility of intra-patient dose escalation of TMZ during maintenance therapy with ABT-888 and TMZ.
4. To describe the toxicities associated with administering ABT-888 and radiation therapy, followed by ABT-888 and TMZ, in patients with newly diagnosed DIPG.
5. To estimate the proportion of newly diagnosed DIPG patients treated on protocol that are determined to have experienced pseudoprogression.

1.2 Phase II Primary Objectives

1. To estimate the overall survival distribution for newly diagnosed patients with DIPG treated with the combination of ABT-888 and radiation therapy, followed by ABT-888 and TMZ, and compare to PBTC historical controls.
2. To study the feasibility of intra-patient dose escalation of TMZ during maintenance therapy with ABT-888 and TMZ.
3. To estimate the proportion of newly diagnosed DIPG patients treated on protocol that are determined to have experienced pseudoprogression.

1.3 Secondary Objectives

1. To estimate the progression free survival distribution and to summarize the best tumor responses observed prior to progression or recurrence.
2. To explore the plasma pharmacokinetics (PK) of ABT-888 during ABT-888 and radiation therapy.
3. To explore peripheral blood mononuclear cell (PBMC) PARP activity before and after treatment with ABT-888.
4. To explore quantifying non-homologous end-joining (NHEJ) activity or γ-H2AX levels (as surrogate markers of unrepaired DSBs) in PBMC before and after treatment with ABT-888.
5. To explore quantifying PARP activity and DNA repair protein levels in biopsied atypical pontine gliomas, if available.
6. To explore associations of molecular parameters from secondary aims 3, 4 and 5 with PFS and OS after conclusion of clinical trial.
7. To explore the quantitative MR measures of relative cerebral blood volume (rCBV), vascular permeability (Ktrans, vp, and ve values), and apparent diffusion coefficient (ADC) within the first six months of initiating protocol treatment to correlate with disease outcome and determine whether such metrics differentiate patients with pseudoprogression from those with true early progressive disease.
8. To explore the potential utility of urine biomarkers as a novel, non-invasive method of detecting and tracking changes in the status of pediatric brain stem gliomas.

2. BACKGROUND

2.1 Study Disease(s)

Pediatric Diffuse Intrinsic Pontine Gliomas

Prognosis for children with diffuse pontine glioma (DIPG) remains dismal and has not changed substantially during the last three decades. The combined 1-year PFS from three recent PBTC clinical trials for 140 patients with newly diagnosed DIPG was 15.9 ± 3.1% (section 13.1), highlighting the continued need for novel treatment approaches in this population. Assuming that these aggressive tumors are generally irradiation resistant, and intensification of traditional chemotherapy agents failed to improve outcome, it is reasonable to hypothesize that novel agents that target a critical tumor-specific pathway mediating irradiation and/or chemotherapy resistance may be worthy of clinical investigation.

2.2 CTEP IND Agent

ABT-888 - Poly(ADP-Ribose) Polymerase (PARP) and DNA Repair

Most chemotherapy drugs induce single-stranded DNA breaks (SSB), and irradiation induces double-stranded DNA breaks (DSB). It is hypothesized that refractory pediatric CNS tumors have enhanced SSB and DSB repair and are therefore resistant to chemotherapy and irradiation, respectively. Repair of SSB is mediated by multiple mechanisms, including the base-excision repair (BER), and DSBs are repaired by the homologous recombination repair (HRR) and non-homologous end-joining (NHEJ) repair pathways. Poly(ADP-ribose) polymerase (PARP) is a critical enzyme that binds to SSBs and DSBs and recruits and activates repair proteins from the BER,[1] NHEJ,[2] and HRR[3] pathways. Elevated PARP expression and/or activity may therefore mediate chemotherapy and irradiation resistance in CNS tumors.

PARP Over-Expression in Pediatric CNS Tumors

We have previously shown that 56 out of 59 pediatric medulloblastomas (MB) and 16 out of 18 pediatric glioblastoma multiforme (GBM) have elevated PARP expression by immunohistochemistry (IHC), compared to absent expression in age-matched normal brain (JM Su and XN Li, unpublished data; data previously summarized in PBTC-027). Other investigators have also demonstrated PARP over-expression in pediatric CNS tumors,[4] including DIPG.[5] These data suggest that elevated PARP activity in pediatric brain tumors may enhance DNA repair after irradiation or chemotherapy, mediate treatment resistance, and serve as a potential therapeutic target for enhancing sensitivity to radiation therapy and chemotherapy.

2.2.1 Preclinical Studies

2.2.1.1 *ABT-888 Enhances Pediatric CNS Tumor Response to Chemotherapy*

ABT-888 is a potent oral PARP inhibitor that potentiates chemotherapy drugs and enhances radiation efficacy in xenograft models of human tumors.[6,7] We previously showed that ABT-888 effectively penetrates the blood-brain barrier (CSF to plasma ratio 57 +/- 7% in non-human primate models)[8] and enhanced *in vivo* tumor response to temozolomide (TMZ) in a pediatric GBM and MB intracranial xenograft models (Figure 1A, 1B; JM Su and XN Li, unpublished data).

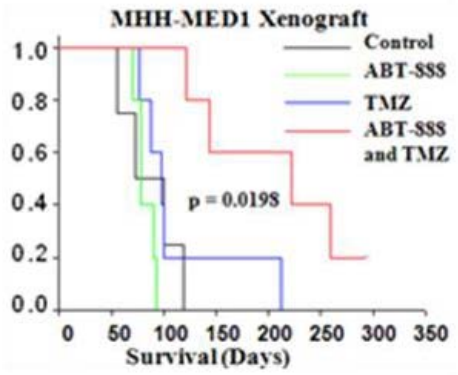

Figure 1A: ABT-888 Enhances TMZ Efficacy in a Pediatric MB Xenograft Models

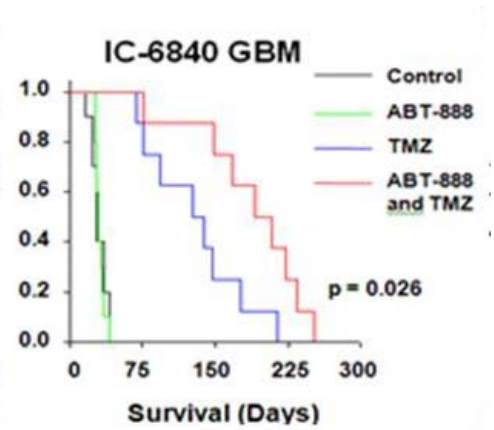

Figure 1B: ABT-888 Enhances TMZ Efficacy in a Pediatric GBM Xenograft Model

2.2.1.2 *Preferential accumulation of ABT-888 in brain tumor versus plasma*

GBM and MB xenografts and neighboring cerebrum or cerebellum were harvested immediately after 5 days of ABT-888 treatment and analyzed for ABT-888 concentrations by liquid chromatography-mass spectroscopy. ABT-888 accumulated preferentially in the xenografts compared to normal neighboring brain, and the tumor to plasma ratio of ABT-888 was 8.25 +/- 6 (n = 10 mice; JM Su and XN Li, unpublished data). This preferential accumulation of ABT-888 in intracranial xenograft tumors was also previously confirmed by Donawho et al.[6] This preferential accumulation of ABT-888 in brain tumors, achieving concentration exceeding 5-fold of the plasma level, is a potential therapeutic advantage in targeting CNS tumors.

2.2.1.3 *ABT-888 Enhances Pediatric MB and GBM Response to Irradiation*

Using primary cultured cells from freshly resected surgical specimens (6 pediatric MB and 5 GBM), we studied the potential of *in vitro* radiation enhancement by ABT-888 treatment. Potential CD133+ tumor stem cells were isolated by flow cytometry and cultured separately from non-stem tumor cells in 96-well plates. We selected an ABT-888 concentration range of 1 to 5 μM, corresponding to plasma concentration of ~ 50 to 244 ng/ml (assuming a tumor to plasma ratio of 5, which we and others have demonstrated in xenograft models), which has been safely achieved in adult and pediatric trials (see data below). Tumor cells were pre-treated with ABT-888 for 24 hours, followed by a single fraction of irradiation, and then drug treatment was continued for 14 days, and cell viability was estimated with cell counting kit 8 (CCK8). ABT-888 treatment resulted in radiation sensitization of either non-stem or stem-like tumor cells in 5 out of 6 MB and 4 out of 5 GBM models (Figure 2).

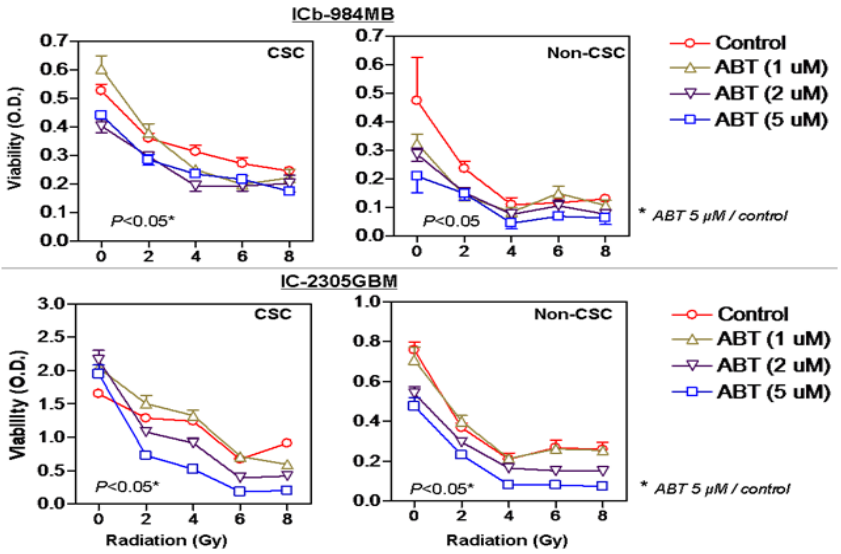

Figure 2: ABT-888 Enhances In Vitro Medulloblastoma and GBM Response to Radiation; CSC = Cancer Stem Cells

Using an intracranial pediatric GBM model established from a fresh surgical tumor specimen,[9] we proceeded to study the *in vivo* radiation potentiation effect of ABT-888. Tumor cells were injected intra-cerebrally as previously described, and treatment was initiated two weeks afterward. The control group received radiation therapy consisting of 2 Gy daily fractions for 5 days, and the test group received ABT-888 by intra-peritoneal injections twice daily, with the morning dose delivered 30-60 minutes prior to daily irradiation. Animals were then observed after 5 days of treatment. We started with ABT-888 at 12.5 mg/kg bid (25 mg/kg/day), the dose that has been shown to optimize chemotherapy potentiation without causing excessive toxicities. While there was a trend toward increasing survival in the group of animals that received ABT-888 and radiation, the result did not achieve statistical significance (data not shown). [redacted] we repeated the *in vivo* experiments described above but with increased dosing of ABT-888 (at 50 mg/kg/day and 100 mg/kg/day). Animals that received ABT-888 (50 mg/kg/day) with radiation showed statistically significant improved survival compared to control animals that received radiation or ABT-888 alone (Figure 3). In the cohort of animals that received ABT-888 at 100 mg/kg/day, those mice that received ABT-888 and radiation also showed improved survival, but the survival curve is not finalized yet (data not shown).

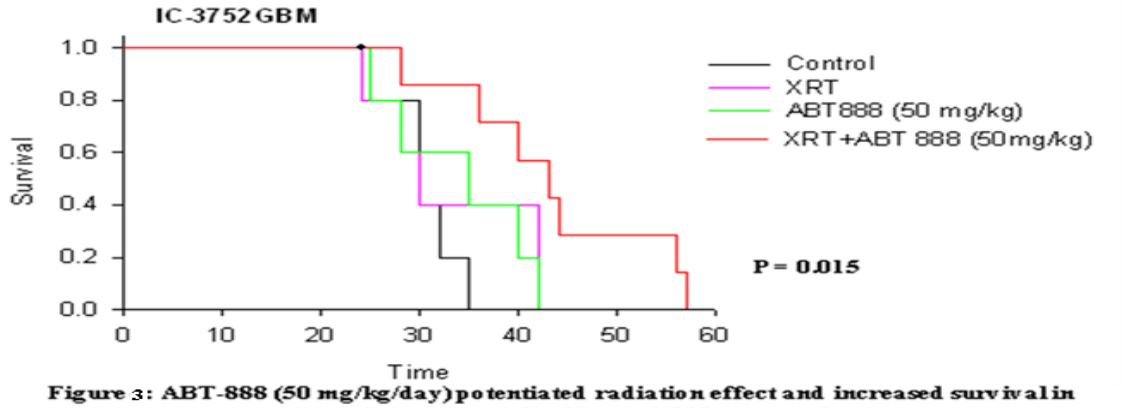

Figure 3: ABT-888 (50 mg/kg/day) potentiated radiation effect and increased survival in mice with intracranial pediatric GBM xenografts.

2.2.2 Adult Clinical Trials

2.2.2.1 *Phase I Trial of ABT-888 and Chemotherapy in Adults*

AbbVie Laboratories has completed a phase I clinical trial of ABT-888 and TMZ in adults with recurrent/progressive solid tumors. [redacted]

2.2.2.2 *Phase I Trial of ABT-888 and XRT in Adults*

AbbVie Laboratories began a phase I clinical trial of ABT-888, TMZ, and XRT, followed by ABT-888 and TMZ for 4 additional courses in adults with newly diagnosed GBM in early 2010 (NCT00770471). [redacted]

AbbVie Laboratories is also completing another phase I study of ABT-888 and whole-brain irradiation in adults with solid tumors metastatic to the brain (NCT00649207). Irradiation is administered in either 15 fractions (2.5 Gy daily) for 3 weeks, for a total of 37.5 Gy, with ABT-888 given bid daily throughout the duration of XRT. No DLT has been observed with ABT-888 at 50 mg or 100 mg bid (6 and 7 patients, respectively), and the trial is continuing with patients receiving ABT-888 at 150 mg BID, with the expectation that this dose level will be declared tolerable by the end of October 2011 ([redacted] Alice Chen, CTEP, personal communication).

2.2.3 Pediatric Studies

2.2.3.1 *Phase l Trial of ABT-888 and TMZ in Children with Recurrent CNS Tumors*

We have completed a phase I clinical trial of ABT-888 and TMZ in children with recurrent/refractory CNS tumors through the Pediatric Brain Tumor Consortium (PBTC-027; NCT00946335). The initial treatment schema was as follows:

Table 1

Dose Levels		
Dose Level	ABT-888 Dose, mg/m^2/dose BID x 5 days	TMZ Dose, mg/m^2/day x 5 days
-1	15 mg/m^2 /dose BID	150 mg/m^2/day
0	15 mg/m^2 /dose BID	180 mg/m^2/day
1 (Starting dose level)	20 mg/m^2 /dose BID	180 mg/m^2/day
2	25 mg/m^2 /dose BID	180 mg/m^2/day
3	30 mg/m^2 /dose BID	180 mg/m^2/day
4	30 mg/m^2 /dose BID	200 mg/m^2/day

Three patients on Dose Level 1 and two patients on Dose Level 0 experienced hematologic DLTs (grade 4 neutropenia and grade 4 thrombocytopenia). Six additional children were enrolled onto Dose Level -1, and only 1 patient had a hematologic DLT (a delay in ANC recovery). One patient developed grade-5 encephalopathy and white matter changes, but these toxicities were ultimately attributed to severe pre-existing hypertension and not related to study drugs. No other DLTs have been observed.

2.2.3.2 *Pharmacology/Pharmacokinetics/Correlative Biological Studies*

Pharmacokinetic data from children enrolled onto PBTC-027 (Table 2) showed that, based on $AUC_{0->12hr}$ and steady state C_{max}, pediatric ABT-888 drug exposure at 15 mg/m^2 is similar to adult dosing at 20 mg bid, and pediatric ABT-888 drug exposure at 20 mg/m^2 bid is slightly lower than adult dosing at 40 mg bid ($AUC_{0->12hr}$ 1.55 µg•hr/mL in 1 child vs 1.87 +/- 0.49 µg•hr/mL in 3 adults). Based on maximally effective $AUC_{0->24hr}$ of 3 µg•hr/mL predicted from animal models and observed $AUC_{0->24hr}$ of 3.74 µg•hr/mL in adults receiving ABT-888 at 40 mg bid, it appears that pediatric dosing of 20 mg/m^2 bid or higher would be required to match the drug exposure seen in adults receiving 40 mg bid. Furthermore, PARP inhibition in PBMCs from children on PBTC-027 was highly variable, averaging only 31.5 +/- 33.6% inhibition in seven patients.

Table 2

Pharmacokinetic Data, PBTC-027 versus Adult Mean ± Standard Deviation				
	PBTC-027 20 mg/m^2	**PBTC-027 15 mg/m^2**	**Adult 40 mg bid (22 mg/m^2)**	**Adult 20 mg bid (11 mg/m^2)**
Number of patients	1	6	3	3
$AUC_{0->12hr}$ (µg•hr/mL)	1.55	1.05 ± 0.42	1.87 ± 0.49	0.99 ± 0.37
Steady State C_{max} (ng/ml)	379	222 ± 64	348 ± 55	171 ± 39

Based on the preliminary PK and pharmacodynamic data from PBTC-027, the trial was amended in an effort to optimize ABT-888 drug exposure and PARP inhibition. The **revised treatment schema** is as follows:

Table 3

Revised Treatment Schema		
Dose Level	ABT-888 Dose, mg/m^2/dose BID x 5 days	TMZ Dose, mg/m^2/dose daily x 5 days
0	15 mg/m^2 /dose BID	135 mg/m^2/day
1 (starting dose level)	20 mg/m^2 /dose BID	135 mg/m^2/day
2	25 mg/m^2 /dose BID	135 mg/m^2/day

Three children treated on revised Dose Level 1 did not have any DLT. One out of 3 children treated on revised Dose Level 2 had grade 4 thrombocytopenia. Another patient on Dose Level 2 had ~40% tumor reduction after 1 course of protocol therapy but also had a symptomatic ischemic infarction within the tumor/brainstem. This complication has not been observed in more than 472 adult patients receiving ABT-888 on various phase I trials to date (CTEP, Alice Chen, personal communication). However, since a possible attribution to ABT-888 could not be completely excluded, three additional patients were enrolled onto Dose Level 1, and no DLTs were observed in the six patients treated on Dose Level 1.

Pharmacokinetic data from children on the revised treatment schema are as follows:

Table 4

Preliminary Pharmacokinetic Data, PBTC-027 versus Adult Mean ± Standard Deviation				
	PBTC-027 20 mg/m^2	**PBTC-027 25 mg/m^2**	**Adult 40 mg bid (~22 mg/m^2)**	**Adult 20 mg bid (~11 mg/m^2)**
Number of patients	3	3	3	3
$AUC_{0->12hr}$ (µg•hr/mL)	1.35 ± 0.16	2.0 ± 0.64	1.87 ± 0.49	0.99 ± 0.37
Steady State C_{max} (ng/ml)	293 ± 58	372 ± 148	348 ± 55	171 ± 39

It appears that children who received ABT-888 at 25 mg/m^2 bid showed PK profile that is essentially identical to adults who received the drug at 40 mg bid, the recommended phase II dose in current adult trials.

PARP inhibition from PBMC from the revised study is as follows:

Table 5

PBTC-027 PARP Inhibition			
Patient	**Dose Level**	**ABT-888 Dose**	**PARP Inhibition**
3541*	1*	20 mg/m^2 bid	10.1%
13182	1	20 mg/m^2 bid	9.1%
14968	1	20 mg/m^2 bid	42.5%
15804	1	20 mg/m^2 bid	38.8%
16816	2	25 mg/m^2 bid	68.9%
17354	2	25 mg/m^2 bid	96.0%
18147	2	25 mg/m^2 bid	47.2%
* Patient 3541 was treated prior to the first amendment and received ABT-888 at 20 mg/m^2 bid with TMZ at 180 mg/m^2			

Although the sample sizes at each dose level are small, it appears that PARP inhibition is greater at 25 mg/m^2 bid versus 20 mg/m^2 bid.

Based on available PK and PARP inhibition data, and with the aim of optimizing drug exposure and biological effect, we amended the PBTC-027 trial to enroll additional patients at Dose Level 2 (ABT-888 at 25 mg/m^2 bid, TMZ at 135 mg/m^2/day, for 5 days every 28 days) with close monitoring for CNS toxicity. Three additional patients were enrolled on Dose Level 2, and no DLTs were observed. Thus, Dose Level 2 (ABT-888 at 25 mg/m^2 bid, TMZ at 135 mg/m^2/day, for 5 days every 28 days) is the recommended phase II dose (RP2D) for children with recurrent or refractory CNS tumors.

To date, a total of twelve patients on PBTC27 have been treated on RP2D, and except for one grade-4 thrombocytopenia and the grade-5 ischemic infarction seen in the first two patients, no other dose-limiting toxicities were reported.

2.3 Rationale

This will be a Phase I/II study of ABT-888 and concurrent radiation treatment, followed by maintenance with ABT-888 and TMZ, in children with newly diagnosed DIPG. Phase I of the study will be the dose-finding portion of the trial to determine the MTD/recommended phase II dose of ABT-888 that can be safely administered with radiation treatment. Dosing of ABT-888 during irradiation will start at 80% of the adult dose (100 mg bid) that has been safely tolerated (section 2.2.2 and 5.1.1.2). After completion of ABT-888 and radiation treatment, patients in Phase I of the study will continue with maintenance therapy, receiving ABT-888 and TMZ at the recommended phase II doses determined from PBTC-027 (section 5.1.3). Intra-patient dose escalation of TMZ will also be studied to optimize TMZ exposure for individual patients (section 5.1.3.1).

The Phase II study will commence once the MTD/recommended phase II dose of ABT-888 that can be safely administered with radiation has been determined from the Phase I study. All patients in the Phase II study will also continue with maintenance therapy, starting with ABT-888 and TMZ at the recommended phase II doses determined from PBTC-027. Intra-patient dose escalation of TMZ will continue to be studied unless excessive toxicities are observed (section 5.1.3.1). In the absence of excessive toxicities or disease progression, all patients can receive up to 10 courses of maintenance therapy. ABT-888 may be available for patients beyond the 10th course of maintenance if the patient is benefiting from the treatment, has at least clinical and radiographic stable disease at the end of course 10 and the investigator and subject agree to continue treatment for up to an additional 13 courses.

2.3.1 Rationale for ABT-888 and Radiation Therapy

Pre-clinical studies have confirmed ABT-888's radiation potentiation in several adult cancers, and we have shown similarly that ABT-888 treatment inhibited DSB repair and enhanced efficacy of radiation in pediatric CNS tumors, including GBM. It is therefore reasonable to study ABT-888 concurrently with irradiation in children with newly diagnosed DIPG tumors.

2.3.2 Rationale for ABT-888 and TMZ

TMZ treatment concurrently with radiation therapy and continuing as maintenance therapy for

six months is now the standard of care for adults with GBM. Although in children with high-grade glioma and DIPG, a COG clinical trial (ACNS0126) of TMZ and irradiation followed by maintenance TMZ has not demonstrated superior survival compared to historical series, we hypothesize that TMZ resistance may account for the lack of therapeutic benefit in the majority of patients. Several pre-clinical studies have demonstrated that PARP inhibition enhanced *in vitro* and *in vivo* TMZ cytotoxicity in multiple malignant glioma cell lines and xenograft models, and such enhanced TMZ cytotoxicity was observed despite enhanced MGMT expression and/or MMR deficiency,[10-12] two of the known tumor mechanisms for TMZ resistance. We have similarly demonstrated that ABT-888 enhanced TMZ efficacy in xenograft models of pediatric GBM. Collectively, available pre-clinical data suggest that PARP inhibition by ABT-888 may reverse TMZ resistance in pediatric gliomas.

2.3.3 Rationale for Targeting Children with Brainstem Gliomas

We and others have shown that PARP is highly expressed in pediatric GBMs and brainstem gliomas. As radiation treatment only leads to a transient response in these aggressive tumors, it is highly likely PARP over-expression, either at the time of diagnosis or in response to irradiation, contributes to apparent radiation resistance. We therefore propose to study the benefit of ABT-888, a PARP inhibitor, in overcoming the radiation resistance in pediatric DIPG.

2.3.4 Rationale for ABT-888 Dosing During Radiation

Although it appears that, from the adult clinical trials, ABT-888 administered concurrently with TMZ and irradiation is associated with dose-limiting hematologic toxicities, administration of ABT-888 concurrently with radiation therapy only has been well tolerated. No DLT was observed at 100 mg BID and cumulative radiation doses of 37.5 Gy and it is anticipated that the dose of 150 mg BID in adults will be declared tolerable by the end of October 2011 ([redacted] AbbVie Laboratories and Alice Chen, CTEP, personal communication). We therefore propose to start at the 80% of the adult dose (100 mg bid) that has been safely tolerated and allow for 2 dose escalations to reach a pediatric dose that is equivalent to 150 mg BID in adults.

2.4 Correlative Studies Background

2.4.1 Rationale for Biology Studies

2.4.1.1 *Potential Mechanism of Resistance against PARP Inhibition*

Pre-clinical studies have shown that ABT-888 induces SSB, collapses replication forks, and results in unrepaired DSB,[13] but tumors capable of enhancing their DSB repair, e.g. by up-regulating HRR, may acquire resistance to PARP inhibition.[14] Conversely, tumors with deficient DSB repair, e.g. BRCA1 or BRCA2 deficient breast or ovarian cancers, should be theoretically hypersensitive to PARP inhibition, whether as a single drug or in combination treatment, and this hypothesis has been confirmed pre-clinically[15] and most recently in two clinical trials.[16,17] Therefore, it is imperative to document the baseline and post-treatment DSB repair capability in tumors, as these molecular markers may predict sensitivity to PARP inhibitors such as ABT-888.

As outlined above, tumors with deficient DSB repair should be hypersensitive to PARP inhibition, whether as a single agent or in combination treatment, and conversely, tumors capable of enhancing their DSB repair may become resistant to PARP inhibition. We therefore propose to study the baseline and post-treatment DSB capability in patients' peripheral blood monocytes

(PBMC) and plan to correlate results retrospectively with clinical outcomes. We also propose to document pre- and post-treatment PARP activity in PBMC as a surrogate measure of ABT-888 biological activity in tumor. In tumors that are atypical appearing and must be biopsied and proven to be high-grade gliomas prior to patients' enrollment, we also propose to quantify PARP activity and DNA repair protein levels (Ku 70, Ku 80, DNA-PK, BRCA 1, BRCA 2, Rad51, and ATM) in either frozen tumors or FFPE samples. These molecular data will help in guiding the design of a future phase 3 clinical trial.

2.4.2 Rationale for Imaging Studies

If ABT-888 is effective in enhancing radiation effect in DIPG as we hypothesize, it is possible that the rate of pseudoprogression may be higher for this proposed study compared to historical series. In parallel with earlier pediatric DIPG trials, for patients showing possible tumor progression on MRI during the first 6 months after the initiation of ABT-888 and irradiation (on required MRI studies performed at weeks 10, week 18, or 26), the treating physician will have the option of allowing the patient to remain on study, continuing protocol therapy, and repeating disease reassessment in 4-6 weeks. Provided that the patient does not show clinical deterioration consistent with tumor progression, has been on a stable or declining dose of steroids, and the subsequent MRI demonstrates tumor regression or stable disease, then the patient will remain on study and continue protocol therapy, and the frequency of subsequent MRI will revert to pre-specified intervals. If the repeat MRI after 4-6 weeks shows disease progression, rather than pseudo-progression, then the time of progression will be the date of the initial MRI, not the follow up scan.

Key to assessing disease course and potential pseudoprogression is inclusion of detailed clinical data documenting each patient's status at the time of scheduled imaging or upon institutional call of "progressive disease." The ability to track neurologic status, symptoms or signs potentially heralding or reflecting progressive disease, or apparent neurologic stability is important in correlating clinical status with imaging findings, and documenting the clinical and/or imaging findings that prompt the investigator to call "progressive disease." We recently documented a relationship between tumor volume and diffusion (measured by serial echoplanar diffusion imaging) prior to and immediately after irradiation in a cross-protocol analysis of prior PBTC newly diagnosed DIPG patients. Tumors with larger diffusion values seemed to respond early to radiation therapy and percent reduction in ADC values correlated with both progression-free and overall survival rates.[18] Tracking MR imaging volumetric, serial findings in the context of a phase II study of tipifarnib and irradiation showed pseudoprogression in just one of the 40 patient's studied.[19]

As a secondary objective, we shall study relative cerebral blood volume (rCBV), vascular permeability (K^{trans}) along with both fractional plasma volume (v_p) and the extravascular volume fraction (v_e), and the apparent diffusion coefficient (ADC) as parameters hypothesized to differentiate pseudoprogression from true early disease progression (PD). These parameters will be studied with serial imaging over the first 6 months after the initiation of ABT-888 and irradiation; quantitative MR metrics will be retrospectively correlated with clinical outcomes to study if they reliably distinguish pseudo-progression from early PD.

Recent preliminary work with adult glioblastoma patients has shown the potential of MR

perfusion and permeability imaging to differentiate between pseudoprogression and true early progression.[20-25] True early progression appears to demonstrate increased relative cerebral blood volume and permeability compared to pseudoprogression.

While there appears to be some controversy regarding the utility of diffusion weighted MR imaging in the context of pseudoprogression, there is evidence to suggest that ADC values can be used to differentiate residual/recurrent tumor from necrosis.[26] This is predicated upon the idea that successful therapy will show increased ADC values secondary to a decrease in cellularity and an increase in the extracellular space.

We hypothesize that in the first 6 months following treatment with ABT-888 and concurrent radiation therapy, pseudoprogression will be marked by significantly lower relative cerebral blood volume (rCBV) compared to that seen in true early progression. T2*-weighted dynamic susceptibility-contrast MR imaging will be acquired to determine serial rCBV values.

In addition, we hypothesize that pseudoprogression will demonstrate significantly lower vascular permeability (K^{trans}) and fractional plasma volume (v_p) compared to cases with true early progression. The lower v_p is postulated to be the result of the absence of angiogenesis that typically characterizes progressive tumor. Extravascular extracellular space volume fraction (v_e) will likely be significantly higher in pseudoprogression versus true early progression. This is postulated to be the result of a decrease in cellularity and an increase in the extracellular space seen in necrosis. T1-weighted dynamic contrast enhanced MR imaging will be used to determine the K^{trans}, v_p, and v_e values serially during the first 6 months after irradiation and ABT-888.

We also hypothesize that pseudoprogression will be associated with significantly higher ADC values compared to those seen in true early progression. Using diffusion tensor MR imaging, we shall determine the ADC values of pseudoprogression and true early progression in the first 6 months following therapy and shall assess ADC values over time using DTI.

Short and long-term effects of irradiation on the brainstem structures will be assessed using diffusion tensor imaging. Previous work has shown that radiation therapy affects the white matter anisotropy.[27-29] Changes have been noted in the brainstem, following treatment for posterior fossa tumors arising outside brainstem itself.[30] Diffusion tensor imaging will be used to assess the fractional anisotropy and ADC values within the tumor, within the white matter of the pons, and within the uninvolved midbrain and medulla.

2.4.3 Rationale for Pharmacokinetic Studies

Based on pre-clinical data demonstrating ABT-888's radiation potentiation in pediatric CNS tumor models and adult phase I clinical data, we will be administering ABT-888 at doses higher than 50 mg/m^2/day (the highest dose studied in PBTC-027) concurrently with radiation therapy. It would be imperative that we characterize PK profile of ABT-888 when given at higher doses in combination with radiation therapy, especially if unanticipated toxicities are encountered. Furthermore, these PK data will be critical in optimal planning of a future phase III study. Therefore, PK studies for ABT-888 during radiation therapy will be **REQUIRED FOR THE PHASE I STUDY**. PK studies for ABT-888 during radiation for the Phase II study will also be performed but will be **optional**.

2.4.4 Rationale for Urine Correlative Study

Despite recent advances in the imaging and treatment of brain tumors, the ability to prospectively screen for new tumors or to detect tumor recurrence remains limited. Clinicians need to know when a tumor is present and – if previously treated – whether recurrence or tumor progression is occurring in order to most effectively direct therapy. For some of the most common pediatric brain tumors, such as medulloblastoma, ependymoma and juvenile pilocytic astrocytoma, patient outcome is closely linked with extent of resection.[31-36] Timely diagnosis of the presence of disease – including recurrence - is of particular importance in the brain, where radical maneuvers to contain tumor spread are often not possible because of eloquent neural tissue. Clinical detection (through observation of neurologic deterioration) of recurrent brain tumors is twice as likely as asymptomatic radiographic recurrence, yet for patients with recurrence detected radiographically before symptoms became present – the patients with early detection who are less likely to have widespread tumor dispersal - had better survival.[37] Thus, earlier detection of recurrence could improve patient outcomes by identifying the mass when it is small, before tumor cells have migrated, increasing the chances for a curative surgical resection.

Unfortunately, certain subtypes of brain tumors remain challenging to treat at any time due to growth within sensitive parts of the brain or intrinsic biologic characteristics conferring resistance to therapy. Yet even with these types of tumors, such as diffuse intrinsic pontine gliomas (DIPG) and glioblastoma multiforme (GBM), the ability to identify tumor status and response to therapy or progression is important so that clinicians provide patients and families with accurate prognostic information.

We therefore propose to study the feasibility of a novel, non-invasive method employing urinary biomarkers to detect the presence, progression and response to therapy of pediatric brain tumors. Studies from our laboratory, now confirmed by others, support the premise that tumor stage and progression may correlate with urinary levels of specific biomarkers,[38,39] specifically that biomarkers related to regulation of vascular stability in the urine of affected patients might represent a novel, non-invasive method of detecting disease status, progression and therapeutic efficacy.[38-42] Our biomarker selection has been based on targeting specific tumor-related molecules and pathways, in contrast to other more general methods of high-throughput proteomic analyses.

Current methods of brain tumor diagnosis and follow-up center around the use of infrequent clinical examinations and expensive radiographic studies, such as computerized tomography (CT) and magnetic resonance imaging (MRI) that often require sedation or anesthesia in children. By contrast, urine collection carries no risks to the patient and is far less expensive (100-fold less than MRIs) so it can easily be done at frequent intervals, potentially enabling earlier detection of recurrent disease and dynamic evaluation of response to therapy. Urine collection is easy, non-invasive, and can be done locally, saving families travel to tertiary care centers. Analysis is rapid, with a turnaround time of 24 hours. Test results are numerical and are compared to specific statistical cutpoints, obviating the need for analysis that relies on subjective measures, such as film review or tissue staining. Additionally, biomarkers provide a method of assessment that relies on metabolic activity; a different – and complementary – approach to the current method of neuroimaging evaluation.

2.4.4.1 *Preliminary data*

We reported the first demonstration of the utility of urinary biomarkers in brain tumors, including the use of molecules specifically related to the regulation of vascular stability[43] and recently published a proof-of-principle case series, that specifically evaluated the ability of a panel of urinary biomarkers such as MMP-2, MMP-9, NGAL and VEGF (See Figure 4 and **Table 6** and **Table 7**).[44] The results of this series support the premise that levels of urinary biomarkers can not only predict the presence of brain tumors but also that they change in response to effective surgical therapy (see Figure 5).

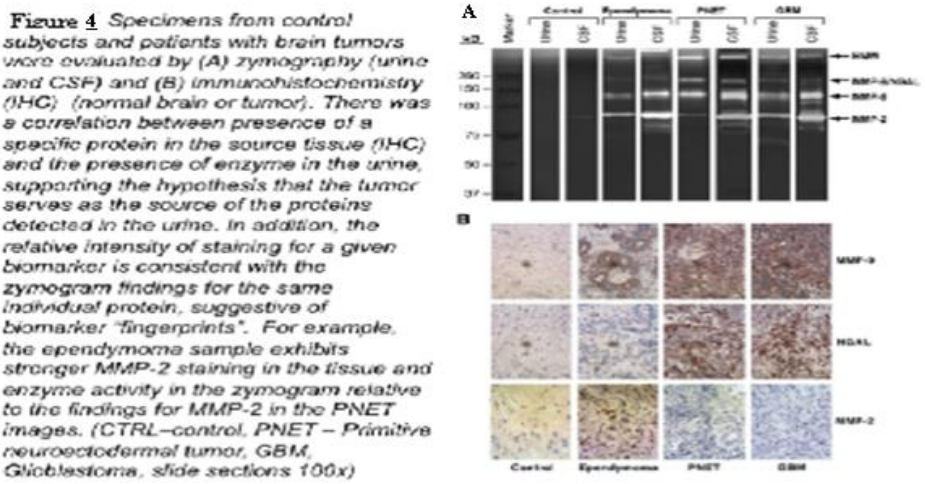

Figure 4 *Specimens from control subjects and patients with brain tumors were evaluated by (A) zymography (urine and CSF) and (B) immunohistochemistry (IHC) (normal brain or tumor). There was a correlation between presence of a specific protein in the source tissue (IHC) and the presence of enzyme in the urine, supporting the hypothesis that the tumor serves as the source of the proteins detected in the urine. In addition, the relative intensity of staining for a given biomarker is consistent with the zymogram findings for the same individual protein, suggestive of biomarker "fingerprints". For example, the ependymoma sample exhibits stronger MMP-2 staining in the tissue and enzyme activity in the zymogram relative to the findings for MMP-2 in the PNET images. (CTRL–control, PNET – Primitive neuroectodermal tumor, GBM, Glioblastoma, slide sections 100x)*

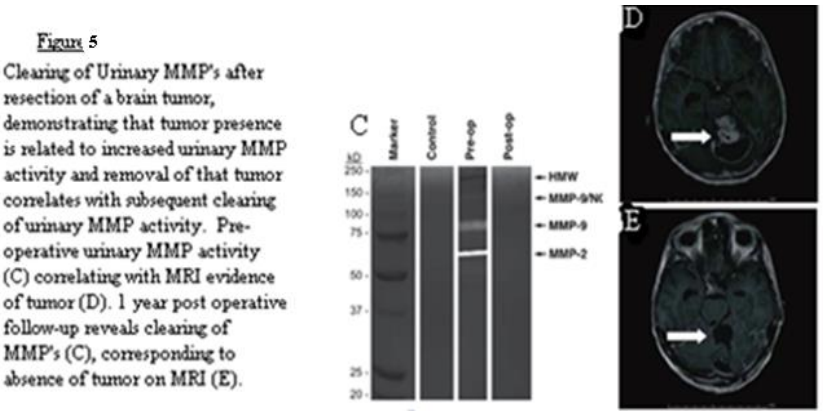

Figure 5
Clearing of Urinary MMP's after resection of a brain tumor, demonstrating that tumor presence is related to increased urinary MMP activity and removal of that tumor correlates with subsequent clearing of urinary MMP activity. Pre-operative urinary MMP activity (C) correlating with MRI evidence of tumor (D). 1 year post operative follow-up reveals clearing of MMP's (C), corresponding to absence of tumor on MRI (E).

We have subsequently identified additional biomarkers that can be detected in urine in association with pediatric brain tumors. These new markers (HB-EGF, EGF, HGF and TIE-2) allow for greater sensitivity in identifying the presence of disease. Specific to this project, these markers have been evaluated in pediatric patients with various subtypes of high grade glioma, including both GBM and anaplastic astrocytoma.

Table 6

Demographics, MMP's and VEGF for Brain Tumor Patients and Controls							
Variable	Control Group (n=23)			Brain Tumor Group (n=28)			*P*
	Median	IQR	Range	Median	IQR	Range	
Age, yr	22	4-55	0-71	32	8-58	1-73	0.37
MMP-9	0	0-0	0-6	0.6	0-5.7	0-294	<0.001*
MMP-9/NGAL	0	0-0	0-12.8	0.8	0.2-1.0	0-1.1	<0.001*
MMP-2	0	0-0	0-3	4.3	0.8-8.8	0-27	<0.001*
VEGF	25	0-250	0-391	753	451-957	25-4462	<0.001*
Female sex, no. (%)	11(48)				13 (46)		0.92

Table 7

Results of Receiver Operating Characteristic (ROC) Curve Analysis and Diagnostic Performance Indices of MMP-2 and VEGF			
Biomarker	AUC	95% CI	*P* value
MMP-2 (ng/ml)	0.894	0.800-0.987	<0.0001
VEGF (pg/L)	0.965	0.913-0.999	<0.0001
Cutoff Points	Sensitivity (95% CI)	Specificity (95% CI)	Accuracy (95% CI)
MMP-2 >0.4 ng/ml	82.1 (65.2-94.0)	95.7 (78.1-99.9)	88.2 (76.1-95.6)
VEGF >350 pg/L	95.2 (76.2-99.9)	89.5 (66.9-98.8)	92.5 (80.0-98.5)

2.4.4.2 *Selection of Brainstem Glioma*

The proof-of-principle data described above suggests that urinary biomarkers may have potential to identify the presence of brain tumors. Further validation of this hypothesis would be bolstered by increasing the number of patients studied and following individual patients longitudinally to evaluate whether changes in disease status (response to therapy or progression) are reflected in corresponding changes in biomarker levels.

This project will focus on urinary biomarker levels in children with brainstem glioma. These patients represent a relatively homogenous population, minimizing variability in age (children), tumor pathology (high grade glioma), location (pontine, without concern for dissemination within the neuraxis) and treatment regimens. As these lesions are not treated with surgical excision, the imprecision inherent to measuring the degree of resection will not be present. The near-uniform progression of these tumors within a short window of time (>80% of patients manifest disease progression within 1 year) allows for data to be collected within a manageable time frame. In combination, these characteristics support the use of this group as an ideal study population.

2.4.4.3 *Justification for Data Collection*

One of the objectives of this study is to identify whether biomarker levels correlate with changes in tumor status. In order to accurately reflect current practice, we will collect clinical data (as measured by Karnofsky and Lansky scores) and several different types of radiographic measures, including changes in size, altered enhancement pattern and the radiologist's overall assessment of tumor status.

Control urines will be obtained from a pre-existing, institutional review board approved urine bank in the Vascular Biology Program at Children's Hospital Boston. These samples come from children with radiographic evaluation of their central nervous system by MRI. Children may

have no evident pathology or evidence of congenital fatty fila (thickening of the terminal end of the spinal cord) or Chiari I malformation (herniation of the cerebellar tonsils below the foramen magnum). No patients have radiographic or clinical histories of tumor, recent trauma, hydrocephalus or arteriovenous malformation (AVM). We currently have control samples from approximately 50 children and collect 1-2 additional samples per week. Thus we anticipate that at the time of analysis we will have a large number of control samples with adequate diversity to enable us to match each clinical sample with an age- and sex-matched control.

2.5 Summary

In conclusion, ABT-888, combined with chemotherapy or radiation, has demonstrated anti-tumor activity in orthotopic xenograft models of pediatric GBM and has shown an acceptable toxicity profile in adult and pediatric phase I trials. Available data suggests that PARP is over-expressed in multiple malignancies, including pediatric high-grade gliomas, and therefore inhibition of this target may overcome chemotherapy and/or radiation resistance. Given the dismal prognosis for children with DIPG, it is appropriate to evaluate the combination of ABT-888 and radiation, followed by maintenance therapy with ABT-888 and TMZ, in this patient population.

3. PATIENT SELECTION

3.1 Eligibility Criteria

The eligibility criteria listed below are to be interpreted literally and cannot be waived. No exceptions will be given. All clinical and laboratory data required to determine eligibility of a patient enrolled on this trial must be available in the patient's medical or research record.

3.1.1 Age

Patients must be ≤ 21 years of age at registration

3.1.2 Tumor

Patients with newly diagnosed diffuse intrinsic pontine gliomas (DIPGs), defined as tumors with a pontine epicenter and diffuse intrinsic involvement of the pons, 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 an anaplastic astrocytoma, glioblastoma multiforme, gliosarcoma, anaplastic mixed glioma or fibrillary astrocytoma.

Note: Patients with juvenile pilocytic astrocytoma, pilomyxoid astrocytoma, gangliogliomas, or other mixed gliomas without anaplasia are not eligible. Patients with disseminated disease are not eligible, and MRI of spine must be performed if disseminated disease is suspected by the treating physician

3.1.3 Neurological Status

Patients must be able to swallow oral medications to be eligible for study enrollment.

3.1.4 Performance Status

Karnofsky ≥50% for patients >16 years of age or Lansky ≥50 for patients ≤ 16 years of age. 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. See **Appendix** A.

3.1.5 Prior Therapy

Patients must have not received any prior therapy other than surgery and/or steroids.

3.1.6 Organ Function

Patients must have normal organ and marrow function documented within 14 days of registration and within 7 days of the start of treatment as defined below:

- Absolute neutrophil count ≥1,000/mm^3
- Platelets ≥100,000/ mm^3 (unsupported)
- Hemoglobin ≥10g/dl (unsupported
- Total bilirubin ≤1.5 times upper limit of normal (ULN) for age
- ALT(SGPT) ≤5 x institutional upper limit of normal for age
- Albumin ≥2g/dl
- Creatinine clearance or radioisotope GFR ≥70 mL/min/1.73 m^2 or
  Serum creatinine based on age/gender as follows:

Table 8

Serum Creatinine for age/gender		
Age	**Maximum Serum Creatinine (mg/dL)**	
	Male	**Female**
1 to < 2 years	0.6	0.6
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.		

3.1.7 Pregnancy Status

Female patients of childbearing potential must not be pregnant or breast-feeding. Female patients of childbearing potential must have a negative serum or urine pregnancy test.

3.1.8 Pregnancy Prevention

Patients of childbearing or child fathering potential must be willing to use a medically acceptable form of birth control, which includes abstinence, while being treated on this study.

3.1.9 Informed Consent

Signed informed consent according to institutional guidelines must be obtained. Assent, when appropriate, will be obtained according to institutional guidelines.

3.2 Exclusion Criteria

3.2.1 Concurrent Illness

Patients with any clinically significant unrelated systemic illness (serious infections or significant cardiac, pulmonary, hepatic or other organ dysfunction), that would compromise the patient's ability to tolerate protocol therapy or would likely interfere with the study procedures or results.

3.2.2 Inability to Participate

Patients with inability to return for follow-up visits or obtain follow-up studies required to assess toxicity to therapy.

3.2.3 Seizures

Patients with active seizures or a history of seizure are not eligible for study entry, with the exception of patients with documented febrile seizure.

3.3 Inclusion of Women and Minorities

Both men and women of all races and ethnic groups are eligible for this trial.

3.4 Treatment at Primary Institution

All experimental protocol therapy should be dispensed and all imaging studies obtained at the primary institution. Laboratory studies, excluding pharmacokinetic and biologic assays, may be performed at a CLIA certified laboratory of the investigator's choice.

3.5 Criteria to Start Therapy

Protocol therapy must begin no later than 30 days after the date of radiographic diagnosis or surgery, whichever is the later date.

All clinical and laboratory studies/assessments required to determine eligibility (excluding diagnosis of the tumor) must be performed within 14 calendar days prior to registration unless otherwise indicated. Imaging evaluations necessary to establish eligibility for study entry must be done within two (2) weeks prior to registration.

Patients must begin therapy no later than 7 calendar days after study registration. If more than 7 calendar days elapse between the date eligibility studies outlined in section 10 were obtained and the start date of treatment, then the following studies must be repeated prior to treatment:

- CBC with differential
- Bilirubin
- ALT (SGPT)
- Serum creatinine

If any of these repeat laboratory studies are outside the parameters required for eligibility or the patient's clinical condition worsens so they no longer meet eligibility requirements (labs may

again be repeated within 48-72 hours), then the patient will not be eligible for protocol therapy.

4. REGISTRATION PROCEDURES

4.1 General Guidelines

4.1.1 Prior to Consent

Prior to consent the protocol's status should be verified via the PBTC Protocol Status web page, to ensure the study is open to accrual.

4.1.2 Informed Consent

Informed consent must be obtained prior to patient registration.

Furthermore, consent should be obtained prior to the initiation of any clinical procedures or assessments performed for the purpose of determining protocol eligibility, which would not otherwise be consistent with the institution's standards of clinical practice.

4.2 Patient Registration

Patients must be registered prior to any protocol treatment. Patient registration is only available to authorized personnel using the PBTC automated registration system. The registration procedures are available in the CRA manual, which is posted on the PBTC members' website. The PBTC Protocol Coordinator may also be contacted at (901) 595-3783 for assistance in the registration process.

Reservations may also be made through the registration system providing time to assess the patient's eligibility. Reservations will be held for a maximum of 7 calendar days by which time the patient must have been registered on study. The patient's reservation should be canceled as soon as it is determined that the patient is not eligible or that the family/patient has decided not to consent to the trial.

5. TREATMENT PLAN

5.1 Agent Administration

Treatment will be administered on an outpatient basis. Reported adverse events and potential risks are described in section 7. Appropriate dose modifications are described in section 6. No investigational or commercial agents or therapies other than those described below may be administered with the intent to treat the patient's malignancy.

All patients will receive ABT-888 plus radiation followed by maintenance therapy with ABT-888 plus TMZ during the Phase I study. The Phase I portion of this trial is a dose finding phase for ABT-888 during the radiation phase of therapy. The Phase II portion of this trial is an efficacy study. Patients will be given a diary (located on the PBTC-033 Protocol webpage) to record the date and time the study drug was taken as well as any comments (e.g. side effects, vomiting, missed doses). If emesis occurs within 10 minutes of administration of ABT-888 or TMZ, the dose should be repeated. A missed dose will not be made up, but the missed dose and reason for missing the dose should be recorded in the diary. The completed Patient dairy will be reviewed weekly during radiation and at each clinic visit during maintenance.

5.1.1 ABT-888 plus Radiation (Phase I)

Patients will receive ABT-888 throughout radiation therapy as outlined in Table 9. Radiation therapy will be administered by 3D-conformal or IMRT to a dose of 54 Gy in 30 daily fractions, treating 5 days per week (typically M-F each week) as outlined in section 5.1.4. Irradiation will not be interrupted for ABT-888 related DLTs unless clinically indicated.

Table 9

Schema – Radiation Phase of Therapy							
Week	1	2	3	4	5	6	Week 7-10
ABT-888, BID	Twice daily, M-F, for 6-7 weeks						Rest/ Evaluation
Radiation Therapy	Daily, M-F, for 6-7 weeks						

ABT-888 will be given twice daily, **Monday through Friday** during radiation therapy (6-7 weeks total). The first dose of ABT-888 should begin on the first day of radiation therapy, except for those children who require sedation for irradiation (see below). **Dosing will be based on the BSA determined at the beginning of radiation therapy.** The morning dose of ABT-888 should ideally be given 60-120 minutes prior to daily radiation treatment. For patients who require sedation for radiation treatment and are not able to take the oral medication due to local requirements for NPO prior to sedation, ABT-888 may be given nightly, and the daytime dose given shortly after the child awakens from sedation, adhering as closely to an every 12-hour schedule as possible. However, if local sedation guidelines permit, then the morning ABT-888 dose should be taken 60-120 minutes prior to radiation. Periodic adjustments in the timing of twice daily ABT-888 administration are allowed if needed to accommodate patient/family convenience provided that the daytime dose is given as close to 60-120 minutes prior to daily irradiation whenever possible and adherence to an every 12-hour dosing schedule is attempted whenever possible.

For patients taking the oral solution, the dose of ABT-888 should be rounded to the nearest 1 mg (e.g. 1.4mg to be rounded to 1.0 mg and 1.5mg to be rounded to 2mg). Specific instructions for handling ABT-888 are provided **Appendix** B. For patients unwilling to take the oral solution of ABT-888, dosing tables for patients with BSA $\geq$ 1.0 m^2 who choose to take only capsules is located in **Appendix** D. Patients are not allowed to take a combination of capsules and the oral solution of ABT-888.

All patients from the Phase I portion of the study, after completing radiation treatment and ABT-888, will continue with maintenance therapy starting week 11, as outlined in section 5.1.3.

5.1.1.1 *Dose-finding Period for the Phase I study*

The observation period for DLTs (Course 1) during Phase I of the study includes the entire duration of radiation therapy (approximately 6 to 7 weeks, allowing for delays in XRT for holidays, mask adjustments, etc.) and the rest period between the end of radiation therapy and the start of maintenance treatment on week 11. Dose limiting toxicities are defined in section 5.2. Guidelines for dose modifications (if required) are provided in section 6.1.

5.1.1.2 *Dose Escalations during ABT-888 and Irradiation*

Dose Level 1 of ABT-888 during radiation treatment will be approximately 80% of the adult dose of 100 mg bid, which has been well tolerated. It is also expected that the 150 mg bid dose level in adults will be declared tolerable shortly. Dose escalation/de-escalation will be done using a standard phase I, 3 + 3 design in increments of approximately 30% (see Table 10 below). If the adult dose of 150 mg bid with radiation has been declared tolerable by the time this trial begins patient accrual, and the starting dose level of 50 mg/m^2 bid is also tolerated in children, we propose to proceed from dose level 1 directly to dose level 3 (85 mg/m^2 bid, equivalent to the adult dose of 150 mg bid). If dose level 3 is not tolerated, then we will de-escalate to dose level 2. If dose level 3 is tolerated, we will study dose level 4 only if supported by adult clinical data and PBMC PARP inhibition data from our ongoing trial.*

Table 10

ABT-888 Dose Levels During Radiation, Phase I of the Study	
Dose Level	**ABT-888 dose (mg/m^2/dose BID), M-F**
0	35 mg/m^2/dose BID
1 (starting dose level)	50 mg/m^2/dose BID
2	65 mg/m^2/dose BID
3	85 mg/m^2/dose BID
4 (pending supporting data)*	110 mg/m^2/dose BID

There will be no intra-patient dose escalation during the irradiation phase of the study (ABT-888 and radiation treatment). Phase I of the study will be completed when MTD/recommended phase II dose of ABT-888 that can be safely administered with radiation has been determined.

5.1.2 Drug Administration during Radiation - Phase II

The Phase II portion of the trial is to evaluate the efficacy of ABT-888 and radiation therapy (at the maximum tolerated or recommended ABT-888 dose level from the Phase I study), followed by maintenance therapy with 10 cycles of ABT-888 and temozolomide in patients with newly diagnosed DIPG. Follow instructions for administering ABT-888 during radiation for the Phase II portion of the study as previously outlined in section 5.1.1.

5.1.3 Drug Administration - Maintenance Therapy (Phase I and Phase II)

Maintenance therapy of ABT-888 and TMZ will begin approximately at week 11 (3-4 weeks after the completion of irradiation and ABT-888) provided that the patient's bone marrow, renal, and hepatic organ functions continue to meet on study criteria. ABT-888 will be given twice daily, with the morning dose given 60-120 minutes prior to the daily dose of TMZ. ABT-888 and TMZ will be given on day 1-5, and each course of treatment will be a minimum of 28 days in length (Table 11).

Table 11

Schema- Maintenance Phase of Therapy						
Each Course	**May receive a maximum of 10 courses**					
Day	1	2	3	4	5	6-28
ABT-888, BID	x,x	x,x	x,x	x,x	x,x	Rest/Evaluation
Temozolomide, once daily	x	x	x	x	x	Rest/Evaluation

All patients (Phase I and Phase II), will start maintenance therapy on week 11 with 25 mg/m^2 bid

of ABT-888 and 135 mg/m^2/day of TMZ (the starting dose for maintenance therapy, Table 12), for 5 days every 28 days, which represent the recommended phase II doses from PBTC-027. **Dosing of ABT-888 and TMZ will be based on the BSA determined prior to each course.** Since this dose determination was based on children with refractory brain tumors, many of whom were heavily pre-treated, it is possible that children with newly diagnosed DIPG, who are chemotherapy naïve at entry, will tolerate higher doses of ABT-888 and TMZ during maintenance.

5.1.3.1 *Intra-patient Dose Escalation of TMZ during Maintenance*

In an effort to maximize the TMZ dose (and potentially the efficacy of the combination treatment) that can be administered with ABT-888 for each patient, **intra-patient dose escalation of TMZ will be studied only for patients with minimal toxicities, considered at least possible related, in each course.** These patients' TMZ dose will be escalated (per Table 12) to 175 mg/m^2/day (Dose Level 2), and subsequently to 200 mg/m^2/day of TMZ (Dose Level 3) if the toxicities observed after 1 course of protocol therapy at each dose level meet the defined criteria. In patients who are not eligible to dose escalate after course 1due to greater than minimal toxicities, there will be no further attempts to escalate the dose of temozolomide. Patients eligible to dose escalate after course 1 but are not dose escalated due to site related issues may start dose escalation in a subsequent course provided they meet all criteria for dose escalation.

Dose escalation of TMZ is **not allowed** if any of the following conditions exist:

Hematological Toxicity

- > Grade 1 thrombocytopenia
- > Grade 2 neutropenia

Non-hematologic Toxicity

- Any grade 4 non-hematologic toxicity
- Any grade 3 non-hematologic toxicity with the exception of:
  - Grade 3 nausea and vomiting of < 5 days
  - Grade 3 elevation of transaminases that returns to levels meeting eligibility criteria within 7 days of study drug interruption and does not recur upon restarting drug.
  - Grade 3 fever or infection of fewer than 5 days in duration
  - Grade 3 hypophosphatemia, hypokalemia, hypocalcemia or
  - hypomagnesemia responsive to oral supplementation
- Any grade 2 non-hematologic toxicity that persists for > 7 days and is considered medically significant or sufficiently intolerable by patients that requires treatment interruption.

Table 12

Intra-Patient Dose Escalation of TMZ during Maintenance Therapy (Days 1-5 per 28 day cycle)		
Dose Level	**ABT-888 Dose**	**TMZ Dose**
0	20 mg/m^2 BID	135 mg/m^2/day
1 (Starting Dose)	25 mg/m^2 BID	135 mg/m^2/day
2	25 mg/m^2 BID	175 mg/m^2/day
3	25 mg/m^2 BID	200 mg/m^2/day

A patient who experience any dose-modifying toxicity (defined in section 6.2) during intra-patient TMZ dose escalation will not be dose-escalated again, and he/she will receive the remaining maintenance therapy at the highest dose level on which no dose-modifying toxicity was observed in that patient (for example, a patient who experiences any dose-modifying toxicity on Dose Level 2 will complete the remainder of maintenance therapy on Dose Level 1). Intra-patient dose escalation to a given dose level will be halted based on similar rules employed in traditional 3+3 designs, i.e. if 2 out of first 2-6 patients 4 out of first 12 patients, etc. experience dose modifying toxicities.

Patient specific dose de-escalations will occur as needed when dose-modifying toxicities arise (section 6.2). Dose level 0, namely ABT-888 at 20 mg/m^2 bid and TMZ at 135 mg/m^2, is provided to allow for one dose de-escalation for patients experiencing dose-modifying toxicities at Dose Level 1. Any patient who experiences dose-modifying toxicities at dose level 0 will be taken off protocol therapy.

5.1.3.2 *Criteria for starting subsequent courses - Maintenance*
Patients will start each subsequent course of maintenance therapy with recovery of ANC ≥1,000/μl and Platelet count ≥100,000/μl.

5.1.4 Radiation Therapy Guidelines

General Guidelines
All patients will receive radiation therapy on this protocol with the targeted volume based on the extent of disease defined by neuroimaging prior to radiation therapy. This study specifies a 1 cm clinical target volume margin and mandates the use of CT-MR registration to define the target volume. The allowed treatment methods are restricted to conformal or intensity-modulated radiation therapy using photons and electronic data submission is required. Proton therapy is not allowed.

Treatment Planning Specifics
The goal of the treatment planning process is to develop a plan to deliver a uniform dose to the planning target volume which includes all known tumor plus the specified clinical target volume margin. The protocol specified total dose is 54 Gy using conventional fractionation. Because the total dose does not exceed the recommended dose limits for the spinal cord and optic chiasm, volume reductions are not required nor recommended.

Required Benchmark and Questionnaires
Radiation therapy will be administered using photons. Required photon methods include 3D conformal radiation therapy (3D-CRT) or intensity modulated radiation therapy (IMRT).Centers participating in this protocol using 3D-CRT are required to complete the 3D benchmark; those using IMRT must complete the IMRT benchmark and questionnaire or phantom. All centers participating in this protocol must complete the QARC CT/MR image fusion benchmark. Benchmark materials and questionnaires may be obtained from the Quality Assurance Review Center (www.qarc.org) and must be submitted before patients on this protocol can be evaluated. For information regarding the IMRT phantoms, please contact the RPC (http://rpc.mdanderson.org/rpc). If IMRT is used, the monitor units generated by the IMRT planning system must be independently checked prior to the patient's first treatment.

Measurements in a QA phantom can suffice for a check as long as the plan's fluence distributions can be recomputed for a phantom geometry.

Guidelines and Requirements for the Use of IMRT
Investigators using IMRT will be required to comply with the guidelines developed for the use of IMRT in National Cancer Institute sponsored cooperative group trials. These guidelines are available through www.qarc.org. These guidelines require that the protocol explicitly state their requirements and methods for localization and immobilization; the use of volumetric imaging; target and organ motion management; nomenclature, definitions, and rationale for targets and organs at risk; target volume coverage and normal tissue dose constraints; effects of heterogeneity in tissues; and quality assurance.

5.1.4.1 *Indications for Radiation Therapy*
All patients enrolled on this protocol will receive concurrent ABT-888 and radiation therapy.

5.1.4.2 *Timing*
All patients should be seen in consultation by a radiation oncologist at the time of study enrollment. The purpose of the consultation is to participate in the initial evaluation and to review the adequacy of the initial diagnostic imaging studies that will be used for subsequent RT planning. If additional imaging studies are pursued, thin section MR (T2-weighted and FLAIR) sequences should be obtained for registration to the CT data set to assist in treatment planning.

Patients are not allowed to have received radiation therapy prior to enrollment on this protocol and urgent irradiation is not envisioned under any circumstance.

The first dose of ABT-888 should ideally be given 60-120 minutes prior to timing of radiation treatment.

5.1.4.3 *Equipment and Methods of Delivery and Verification*

- Beam Energy

For 3D-CRT, photon energy of > 4 MV shall be used. For IMRT, photon energy from 4 to 10 MV shall be used.

- Treatment Planning

CT (volumetric) based planning is required to optimize dose to the PTV while protecting normal tissues. Organs at risk within the irradiated volume should be contoured including those required. A DVH is necessary to determine target coverage and evaluate dose to normal tissues. CT section thickness should be < 5 mm although 2-3 mm is preferred.

- In-room verification of spatial positioning

Portal imaging is the most common system used to verify patient position, in particular when the target volume is believed to possess a fixed spatial relationship with visualized bony anatomy. Orthogonal paired (AP and lateral) portal images (MV or kV) are required for IMRT and 3D-CRT to verify that the isocenter is in correct alignment relative to the patient position.

Volumetric imaging is allowed in this study. This includes in room kV or MV cone beam or conventional CT imaging. Please submit representative axial images showing the isocenter and the correct alignment in relationship to the patients' position. For CT tomography where

isocenters are not used, a printout of the isodoses overlaid on the fused CT images can be printed to demonstrate in room verification.

5.1.4.4 *Target Volumes*

- General Comments

International Commission on Radiation Units and Measurements (ICRU) Reports 50, 62 and 78 (www.icru.org) define prescription methods and nomenclature that will be utilized in this study. MRI obtained immediately prior to RT will be utilized for treatment planning. MR pre- and post-gadolinium T1 and T2 sequences shall be reviewed. Usually the T2 weighted images are used to determine target volumes but a combination of T2 and FLAIR can be used. The GTV, CTV, PTV and normal tissues must be outlined on all axial imaging slices on which the structures exist.

- Definition of GTV, CTV and PTV

Gross tumor volume (GTV) is based on the most recent MRI. The GTV is defined as the abnormal signal on MRI (usually T2 weighted, but a combination of T2 or FLAIR abnormality on the appropriate MR sequence). Investigators should register the immediate pre-irradiation MR imaging sequences that demonstrated tumor and contour the GTV.

The clinical target volume (CTV) includes the GTV with an added margin that is meant to treat subclinical microscopic disease and is anatomically confined (i.e. the CTV is limited to the confines of the bony calvarium, falx and tentorium where applicable or extends up to but not beyond neuroanatomic structures through which tumor extension or invasion is certain not to have occurred). The CTV may be manually moved inward to the inner table of the bony calvarium. For diffuse pontine gliomas, spread usually occurs to the confines of the brainstem circumferentially, rostral to the midbrain and distal to the medulla. The CTV margin will be 1.0 cm for all patients, with most of the CTV growth superiorly and inferiorly as the CTV will not be beyond the brainstem circumferentially. The CTV margin chosen for this study requires treatment planning MR and/or diagnostic MR imaging data with image section thickness ≤ 5mm.

The planning target volume (PTV) includes a margin which is added to the CTV in 3-dimensions to create the PTV. It is geometric and not anatomically defined. The PTV has 2 components, the internal margin (IM) and the set-up margin (SM). The IM is meant to compensate for all movements and variations in size and shape of the tissues contained within the CTV. The SM is meant to account for set-up, mechanical and dosimetric uncertainties related to daily patient positioning, treatment equipment and software. For this study, the PTV margin should be 3 or 5 mm. The use of PTV margin of 3 mm requires written documentation that image guided radiation therapy (IGRT) methods are used on a daily basis or alternatively a head fixation system or verification system was used with weekly imaging. For this study, IGRT is defined as 2- or 3-dimensional digital imaging positioning. For all other cases, a PTV margin of 5 mm shall be used. Given that the CTV is generally confined to the intracranial space, the PTV may extend into or beyond bone but is unlikely to extend beyond the surface of the patient. The PTV margin chosen by the treating investigator requires treatment planning MR and/or diagnostic MR imaging data with imaging section thickness ≤ the chosen PTV margin.

5.1.4.5 *Target Dose*

- Dose Definition

Photon dose is specified in centigray (cGy)-to-muscle.

- Prescribed Dose and fractionation

Planning Target Volume: The total dose to the PTV will be 5400 cGy administered in 30 fractions of 180 cGy.

- *Dose Uniformity*

At least 95% of the protocol-specified dose should encompass 100% of the PTV. No more than 10% of the PTV should receive greater than 110% of the protocol dose as evaluated by DVH. The 100% isodose line should be equal to the protocol specified dose. Wedges, compensators and other methods of generating more uniform dose distributions are encouraged.

- *Tissue heterogeneity*

Calculations must take into account tissue heterogeneity and should be performed with CT-based treatment planning to generate dose distributions and treatment calculations from CT densities. For questions about heterogeneity corrections or approved algorithms, please contact QARC (www.qarc.org).

- Treatment Interruptions, Delays and Dose Modifications

The patient should be treated with one fraction per day, 5 days per week. All fields shall be treated each day. There will be no planned rests or breaks from treatment, and once radiation therapy have been initiated, treatment will not be interrupted except for life threatening infection or severe hematological toxicity during the course of treatment. The reason for any interruptions greater than 3 treatment days should be recorded in the patient treatment chart and submitted with the QA documentation. There should be no modifications in dose fractionation due to age or field size.

5.1.4.6 *Treatment Technique*

- Beam configuration

Every attempt should be made to minimize dose to organs at risk without compromising coverage of the target volume. 3DCRT (coplanar or non-coplanar) or IMRT are required to minimize dose to normal tissues.

- Field Shaping

Field shaping for photons will be done with either customized cerrobend blocking or multileaf collimation.

- Simulation

Patient positioning and immobilization device- Reproducible set-up is critical and the use of an immobilization device is to be used. The imaging studies should provide a clear assessment of the target volume with the patient in the treatment position. Anesthesia or sedation may be required in some patients to prevent movement during simulation and daily treatments.

- Special Considerations

Anesthesia or sedation may be required in certain patients, such as very young patients, to prevent movement during simulation and daily treatments.

5.1.4.7 *Organs at Risk*

The organs at risk (OAR) guidelines in this section are recommendations. If the recommended doses to the OAR are exceeded because of target volume coverage requirements or other conditions, an explanation should be included in the quality assurance documentation. In some cases, IMRT may be the preferred treatment method to meet these requirements and the required target volume coverage guidelines.

Dose Constraints:

- Right and Left Optic Nerves and Chiasm

D90% < 1000cGy, D50% < 5400cGy and D10% < 5600cGy – Goal
D90% < 5400cGy, D50% < 5600cGy and D10% < 5800cGy – Maximum
Comment – Effort should be made to avoid direct treatment of the optic nerves and chiasm without compromising target volume coverage. In the event that the recommended maximum dose constraints provided in this section would be exceeded, the treating radiation oncologist may use their discretion to reduce target volume coverage.

Structure definition – The optic nerve may be contoured on CT or MR. The contour should appear on at least two successive CT or MR images.

- Optic Globes

D90% < 500cGy, D50% < 1000cGy and D10% < 3500cGy – Goal
D90% < 1000cGy, D50% < 2000cGy and D10% < 5400cGy – Maximum
Comment – Effort should be made to avoid direct treatment of the anterior chamber of the eye and minimize dose to the entire eye without compromising target volume coverage. In the event that the recommended maximum dose constraints provided in this section would be exceeded, the treating radiation oncologist may use their discretion to reduce target volume coverage.

Structure definition - Each eye should be separately contoured on the treatment planning CT or MR as a circular structure from the most superior to inferior aspect.

- Spinal Cord

D90% < 300cGy, D50% < 2600cGy and D10% < 5700cGy – Goal
D90% < 900cGy, D50% < 5000cGy and D10% < 5900cGy – Maximum
Comment – Effort should be made to minimize dose to the spinal cord without compromising target volume coverage.

Structure Definition - For the purposes of this study, the upper aspect of the spinal cord begins at the inferior border of the foramen magnum and should be contoured on the treatment planning CT. For purposes of comparison and consistency with dose volume data, the spinal cord should be contoured on a number of images to be determined by the image section thickness (CT section thickness, n=number of images; 2mm, n=30; 2.5 mm, n=24; 3 mm, n=20). Using these guidelines, only the superior-most 6cm of anatomic spinal cord is contoured.

- Cochleae

D50% < 3500cGy – Goal (each cochlea)
D50% < 2000cGy – Preferred (each cochlea)
Comment – There is no dose limit for the cochleae.

Structure definition - Each cochlea will be contoured on the treatment planning CT as a circular structure within the petrous portion of the temporal bone. The contour should appear on at least two successive CT images.

5.1.4.8 *Dose Calculation and Reporting*

- Prescribed Dose

The dose prescription and fractionation shall be reported on the RT-1/IMRT Dosimetry Summary Form. The total dose delivered shall be reported on the RT-2 Radiotherapy Total Dose Record. If IMRT is used, the monitor units generated by the IMRT planning system must be independently checked prior to the patient's first treatment. Measurements in a QA phantom can suffice for a check as long as the patient's plan can be directly applied to a phantom geometry.

- Normal Tissue Dosimetry

The dose to the critical organs indicated should be calculated whenever they are directly included in a radiation field. The total dose shall be calculated and reported on the RT-2 Radiotherapy Total Dose Record form. The appropriate dose-volume histograms should be submitted. If IMRT is used for the primary tumor, a DVH must be submitted for a category of tissue called "unspecified tissue," which is defined as tissue contained within the skin, but which is not otherwise identified by containment within any other structure. DVH data for the following organs is required: Total Brain, Optic Nerves (Left and Right), Optic Chiasm, Brainstem, Spinal Cord, Cochleae (Left and Right), Unspecified Tissue

5.1.5 Radiation Quality Assurance

Within a week after completion of RT, detailed treatment data shall be submitted for review.

5.1.5.1 *External beam Treatment Planning System*

- Digitally reconstructed radiographs (DRR) or simulator films for each treatment field and orthogonal (anterior/posterior and lateral) images for isocenter localization for each group of concurrently treated beams. When using IMRT, orthogonal isocenter images are sufficient.

- Isodose distributions for the treatment plan in the axial, sagittal and coronal planes at the center of the treatment or planning target volume. The planning target volume, isocenter and the normalization method must be clearly indicated.

- Dose volume histograms (DVH) for all target volumes and required organs at risk. A DVH shall be submitted for the organs at risk specified in section 5.1.4.8. When using IMRT, a DVH shall be submitted for a category of tissue called "unspecified tissue." This is defined as tissue contained within the skin, but which is not otherwise identified by containment within any other structure.

- Treatment planning system summary report that includes the monitor unit calculations, beam parameters, calculation algorithm, and volume of interest dose statistics. Beams-eye-view (BEV) of portals showing collimator, beam aperture, target volume and critical structures are required when not using IMRT.

5.1.5.2 *Digital Data*

Submission of the treatment plan in digital format is required. Please refer to www.QARC.org and click on "Digital Data" for guidelines regarding digital submission. All submissions, including those that are digital, require hard copy submission of the other items included in this list. If there are any problems with digital data submission, please contact QARC.

5.1.5.3 *Supportive Data*

All diagnostic imaging used to plan the target volume. This includes CT or MRI PRIOR to attempted surgical resection of the primary tumor. Digital format is preferred.

Radiotherapy record (treatment chart) including prescription and daily and cumulative doses to all required areas and organs at risk. Documentation of an independent check of the calculated dose when IMRT is used should be included. If the recommended doses to the organs at risk are exceeded, an explanation should be included for review by the QARC and the radiation oncology reviewers.

If a PTV margin of 3 mm is used, written documentation that image-guided radiation therapy (IGRT) methods are used on a daily basis or alternatively that a head fixation system or verification system was used with weekly or more frequent imaging. See section 5.1.4.4.

Forms
RT-1/IMRT Dosimetry Summary Form.
RT-2 Radiotherapy Total Dose Record Form.

These data should be forwarded to:
Quality Assurance Review Center
640 George Washington Highway Suite 201
Lincoln, Rhode Island 02865-4207
Phone: (401) 753-7600
Fax: (401) 753-7601

Questions regarding the dose calculations or documentation should be directed to:
COG Protocol Dosimetrist
Quality Assurance Review Center
640 George Washington Highway Suite 201
Lincoln, Rhode Island 02865-4207

5.1.5.4 *Definitions of Deviations in Protocol Performance*

Table 13

	DEVIATION	
	Minor	**Major**
Prescription Dose	Difference in prescribed or computed dose is 6-10% of protocol specified dose	Difference in prescribed or computed dose is > 10% of protocol specified dose
Dose Uniformity	>10% PTV received > 110% of the prescription dose *or* 95% isodose covers < 100% of CTV	90% isodose covers < 100% of CTV
Volume	PTV and CTV margins are less than the protocol specified margins in the absence of anatomic barriers to tumor invasion or without written justification	GTV does not encompass MR visible tumor
Organs at Risk	Dose to any OAR exceeds the goal dose stated in section 5.1.4.7	Dose to any OAR exceeds the maximum dose stated in section 5.1.4.7

5.2 Definition of Dose-Limiting Toxicity (DLT)

Toxicities will be graded according to the NCI Common Terminology Criteria for Adverse Events v4.0 (CTCAE) http://ctep.cancer.gov/reprting/ctc.html. Dose limiting toxicities (DLT) will be defined as any of the following events that are at least (possibly, probably, or definitely) attributable to ABT-888 observed during the dose finding phase (the first 10 weeks of therapy).

5.2.1 Dose-Limiting Toxicities during Phase I

During the Phase I portion of the study, the observation period for DLTs includes the entire duration of radiation therapy (approximately 6 to 7 weeks, allowing for delays in XRT for holidays, mask adjustments, etc.) and the rest period between the end of radiation therapy and the start of maintenance treatment (week 1 through week 10). All dose modifications are to follow the guidelines provided in section 6.1.

5.2.1.1 *Interruption of Radiation Treatment*

Any interruption of irradiation, related to protocol therapy and lasting 5 consecutive days or 10 cumulative days during irradiation is defined as a DLT.

5.2.1.2 *Non-Hematologic Dose Limiting Toxicity*

- Any grade 4 non-hematologic toxicity
- Any grade 3 non-hematologic toxicity with the exception of:
  - Grade 3 nausea and vomiting of < 5 days
  - Grade 3 elevation of transaminases that returns to levels meeting eligibility criteria within 7 days of study drug interruption and does not recur upon restarting drug
  - Grade 3 fever or infection of fewer than 5 days in duration
  - Grade 3 hypophosphatemia, hypokalemia, hypocalcemia or hypomagnesemia responsive to oral supplementation
- Any grade 2 non-hematologic toxicity that persists for > 7 days and is considered medically significant or sufficiently intolerable by patients that requires treatment interruption.

5.2.1.3 *Hematological Dose Limiting Toxicity*

- ≥ Grade 3 thrombocytopenia
- Grade 4 neutropenia

5.2.1.4

Any ABT-888 related adverse event during the observation period for DLTs that leads to a dose reduction or results in the permanent cessation of therapy will be considered dose limiting.

5.3 General Concomitant Medication and Supportive Care Guidelines

Record the use of all concomitant medications listed in this section.

5.3.1 Steroids

Corticosteroids should be used at the lowest dose to control symptoms of edema and mass effect, and discontinued, if possible.

5.3.2 Anticonvulsants

Anticonvulsants should be used, if indicated.

5.3.3 Growth Factors

Growth factors that support white cell count or function can only be administered as outlined in sections 6.1 and 6.2 or for documented infection and/or sepsis.

5.3.4 Anti-emetics

Patients may receive daily prophylactic ondansetron or granisetron during the radiation phase (receiving ABT-888 and radiation) per local practice. During maintenance therapy, patients should receive prophylactic ondansetron or granisetron during the 5-day course of ABT-888 and TMZ, and such treatment may be continued beyond the 5-day period, if clinically indicated. Corticosteroids should not be used as anti-emetics. The use of additional anti-emetics will be at the treating physician's discretion.

5.3.5 Febrile neutropenia

Febrile neutropenia should be managed according to the local institutional guidelines.

5.3.6 Concomitant Therapy

Other anti-cancer or experimental agents or therapies are not permitted.

5.4 Duration of Therapy

In the absence of treatment delays due to adverse event(s), treatment may continue for 10 cycles. See section 2.4.2 or 13.2.1 for guidelines on possible pseudo-progression on MRIs performed at weeks 10, 18, and 26. All patients will conclude protocol based therapy at the completion of 10 cycles of maintenance treatment. At the end of protocol defined therapy, the patient should complete all end of treatment assessments.

5.4.1 Extended Therapy

ABT-888 may be available for patients beyond the 10^{th} course of maintenance if the patient is benefiting from the treatment, has at least clinical and radiographic stable disease at the end of course 10 and the investigator and subject agree to continue treatment for up to an additional 13 courses (total maximum duration of maintenance therapy is 23 courses). During this therapy, all study specific assessments will cease and procedures consistent with good clinical care will continue. Patients will be followed during this additional period for dates of drug administration, adverse events, laboratory results, date of disease progression, date of last follow up and date of death.

5.5 Duration of Follow Up

The "Off Treatment Date" is to be recorded in the eCRF and is to be consistent with the reason given for going off treatment. Date of "off treatment" must be the greatest of the date of last treatment and date of procedure, date of patient assessment or notification of patient/family decision, or decision made by the physician that resulted in the patient being taken off protocol treatment. The reason for discontinuation of treatment must be documented by the attending investigator in the medical record and recorded in the eCRF.

The "Last Treatment Date" is also to be recorded via the "Off Treatment and Off Study" eCRF and is defined as the last date that the patient received protocol based therapy. The "Off Treatment Date" and the "Last Treatment Date" should be the same for patients that complete all protocol defined treatment (see sections 7, 5.5.2 and the Required Data and Timetable Submission from located on the 033 protocol webpage).

5.5.1 Patients will be considered Off Treatment for the following reasons:

- Development of unacceptable toxicity as outlined in 5.2 or 6.2. See section 5.5.2 for specific reporting requirements.
- Progressive disease (PD), as outlined in section 11.1.4
- Development of a medical or psychiatric illness, that in the investigator's judgment renders the patient incapable of further therapy on this protocol.
- The patient, parent or legal guardian refuses further treatment on this protocol.
- Completion of all protocol defined treatment.
- Pregnancy
- Non-compliance

Patients who are off protocol therapy must be followed until an "Off Study Criterion" is met.

5.5.2 Off-Treatment Data Submission Schedule

Patients will be followed for the resolution of all toxicities considered at least possibly related to ABT-888 occurring while on treatment and for 30 days after the last administration of study drug. Patients will be followed for 3 years from the initiation of protocol treatment for the monitoring of unexpected later developing toxicities or other morbidity and to document disease progression and survival. Data should be updated quarterly in the PBTC RDC database.

For patients who are eligible for extended therapy (5.4.1) they will be followed during this additional period for dates of drug administration, adverse events that are possibly, probably or definitely related to study drug, laboratory results, date of disease progression, and date of last follow up and date of death. At the completion of the additional 13 courses of therapy the patient is considered to have completed protocol therapy and should be taken off treatment (per protocol section 5.5). At the completion of the appropriate follow up period (per protocol section 5.6), the patient should be considered off study.

5.6 Criteria for Removal from Study

The date and reason for the patient coming off study must be documented in the eCRF and the Operations and Biostatistics Center must be notified according to standard reporting guidelines (see sections 7, 5.6.2 and the Required Data and Timetable Submission from located on the 033 protocol webpage).

5.6.1 Patients will be considered Off Study for the following reasons:

- Patient determined to be ineligible.
- Parent, patient, or guardian withdraws consent for continued participation.
- Patient death while on study. The IRB, Investigational Drug Branch (IDB), Study Chair and OBC must be notified as per section 7.
- Patient has been followed for 3 years from the initiation of protocol treatment.

5.6.2 Off-Study Data Submission

No data will be collected documenting treatment or reporting events or disease status that occurs subsequent to the official "off study" date with the exception of adverse events with an attribution of possible, probable, or definite that occur after the "off study" date for agents being studied under a CTEP IND (see section 7).

6. DOSING DELAYS/DOSE MODIFICATIONS

The study chair must be notified of any proposed dose modifications. After consultation with the Study Chair, the site should follow the dose modification guidelines in this section.

6.1 Dose Modification for DLT during ABT-888 and Radiation

6.1.1 Non-Hematologic

For ABT-888-related non-hematologic DLTs during radiation therapy, ABT-888 will be withheld until the toxicity resolves to meet on study parameters (section 3.1.6), and the patient will restart ABT-888 at one dose level lower (see **Table 10**), if not already at Dose Level 0. Patients who experience a non-hematologic DLT on Dose Level 0 will complete radiation without ABT-888.

6.1.2 Hematologic

For ABT-888-related hematologic DLTs, ABT-888 will be withheld until criteria described below are met, and ABT-888 will be restarted at one dose level lower, if not already at Dose Level 0. If the same DLT is experienced at the lower dose level, then ABT-888 should be discontinued. If a different DLT is encountered, then the patient may restart ABT-888 at the next lower dose level, if not already at Dose Level 0.

In the event of hematological DLT, CBC should be checked twice weekly until recovery of ANC $\geq 1{,}000/mm^3$ and platelet $\geq 100{,}000/mm^3$. A patient who experiences any DLT despite being treated at Dose Level 0 will complete radiation therapy without ABT-888. **Irradiation should be continued despite ABT-888-related DLT, unless there is a clinical contraindication.**

a. ≥ Grade 3 thrombocytopenia: Platelet transfusion is permissible and strongly encouraged for patients with dose-limiting thrombocytopenia to minimize the risk of intra-tumoral hemorrhage. ABT-888 should not be restarted until platelet count has recovered to $\geq 100{,}000/mm^3$ (transfusion independent). **Radiation therapy should not be interrupted unless clinically indicated.**

b. Patients with grade 4 neutropenia may receive filgrastim (G-CSF) support only for documented infection and/or sepsis, and they should re-start ABT-888 at the next lower dose level without GCSF support. ABT-888 should not be restarted until the ANC is $\geq 1{,}000/mm^3$ for at least 48 hours without G-CSF support. **Radiation therapy should not be interrupted unless clinically indicated.**

Patients who experience ABT-888-related DLTs during irradiation will still receive ABT-888 during maintenance therapy, provided that they meet the criteria to start subsequent course of

therapy and all prior ABT-888-related toxicities have resolved to baseline.

6.2 Dose Modification for ABT-888 and TMZ during Maintenance

Dose modifying toxicities will be defined as any of the following events that are at least (possibly, probably, or definitely) attributable to ABT-888 and TMZ**.** Dose de-escalation during maintenance should occur if any of the following dose modifying toxicities are present:

6.2.1 Non-Hematologic Toxicity

- Any grade 4 non-hematologic toxicity
- Any grade 3 non-hematologic toxicity with the exception of:
  - Grade 3 nausea and vomiting of < 5 days
  - Grade 3 elevation of transaminases that returns to levels meeting eligibility criteria within 7 days of study drug interruption and does not recur upon restarting drug.
  - Grade 3 fever or infection of fewer than 5 days in duration
  - Grade 3 hypophosphatemia, hypokalemia, hypocalcemia or hypomagnesemia responsive to oral supplementation
- Any grade 2 non-hematologic toxicity that persists for > 7 days and is considered medically significant or sufficiently intolerable by patients that requires treatment interruption

For any of the above non-hematologic toxicities during maintenance therapy, ABT-888 and TMZ will be withheld until the toxicity resolves to meet on study parameters (section 3.1.6), and the patient will restart ABT-888 and TMZ at the next lower dose level (see **Table 12**). Protocol therapy will be discontinued if a non-hematologic dose modifying toxicity occurs at dose level 0.

6.2.2 Hematological Toxicity

- ≥ Grade 3 thrombocytopenia
- Grade 4 neutropenia
- A delay of > 14 days in starting subsequent cycles of maintenance due to ANC < 1,000/mm^3 and/or platelet < 100,000/mm^3 (for example, a patient never experienced grade 3 thrombocytopenia, but his/her platelet count lingered between 50,000 to 100,000/ mm^3 beyond 42 days after the start of the previous course of maintenance therapy)

For the hematologic toxicities outlined above that occur during maintenance therapy, ABT-888 and TMZ will be withheld until criteria described below are met, and ABT-888 and TMZ will be restarted at the next lower dose level (see **Table 12**). Protocol therapy will be discontinued if a hematologic dose-modifying toxicity occurs at dose level 0.

a. ≥Grade 3 thrombocytopenia: Platelet transfusion is permissible and strongly encouraged for patients with dose-limiting thrombocytopenia to minimize risk of intra-tumoral hemorrhage. ABT-888 and TMZ should not be restarted until platelet count has recovered to ≥ 100,000/mm^3 (transfusion independent).

b. Patients with grade 4 neutropenia may receive filgrastim (G-CSF) support only for documented infection and/or sepsis. ABT-888 and TMZ should not be restarted until the

ANC is $\geq$ 1,000/mm^3 and for at least 48 hours without G-CSF support. Patients should start the subsequent cycle at the next lower dose level, without G-CSF support.

Complete blood counts should be repeated twice weekly for any hematological toxicity described above until counts recover to meet criteria for starting subsequent course of maintenance therapy.

7. ADVERSE EVENTS: LIST AND REPORTING REQUIREMENTS

Adverse event (AE) monitoring and reporting is a routine part of every clinical trial. The following list of AEs (section 7.1) and the characteristics of an observed AE (section 7.2) will determine whether the event requires expedited reporting (via CTEP-AERS) **in addition** to routine reporting.

- Baseline Abnormalities
  Any baseline (pretreatment) abnormalities observed during the initial physical examination should be recorded in the PedBraTum database.

- Routine AE Recording
  Only record adverse events grades 1 and 2 if the attribution is at least possibly related to ABT-888. Record all adverse events grades 3 through 4 and deaths (while on treatment or within 30 days of treatment), regardless of attribution on the electronic case report forms (PedBraTum RDC database).

7.1 Comprehensive Adverse Events and Potential Risks List (CAEPR)

The Comprehensive Adverse Event and Potential Risks list (CAEPR) provides a single list of reported and/or potential adverse events (AE) associated with an agent using a uniform presentation of events by body system. In addition to the comprehensive list, a subset of AEs, the Specific Protocol Exceptions to Expedited Reporting (SPEER), appears in a separate column and is identified with ***bold*** and ***italicized*** text. The SPEER is a list of events that are protocol-specific exceptions to expedited reporting to NCI via CTEP-AERS (except as noted below). Refer to the 'CTEP, NCI Guidelines: Adverse Event Reporting Requirements' http://ctep.cancer.gov/protocolDevelopment/electronic_applications/adverse_events.htm for further clarification.

NOTE: The highest grade currently reported is noted in parentheses next to the AE in the SPEER. Report ONLY AEs higher than this grade expeditiously via CTEP-AERS. If this CAEPR is part of a combination protocol using multiple investigational agents and has an AE listed on different SPEERs, use the lower of the grades to determine if expedited reporting is required.

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7.1.2 Adverse Event List for Temozolomide

Table 14

Known Toxicities of Temozolomide			
	Likely (≥20%)	Less Likely (≤20%)	Rare but Serious (<5%)
Immediate: within 1-2 days of receiving drug	Anorexia Constipation Nausea Diarrhea	,Abdominal pain, Headache, Itching, Rash, Urinary frequency and/or infection, Vomiting, Dizziness, Anxiety, Confusion	Ataxia, Convulsions, Dysphagia, Hemiparesis, Thromboembolism

Prompt: Within 2-3 weeks, prior to next course	Myelosuppression	Lethargy, Mucositis, Peripheral edema; Fever (associated with low neutrophil count), Weight gain, Back pain, Breast pain, peripheral neuropathy, Upper respiratory infection, cough, sore throat, Myalgia, Amnesia, Depression, Visual changes, Insomnia	Myelosuppression for prolonged period with increased risk of infection or death; Hepatic failure
Delayed: Anytime later during therapy, excluding the above conditions		Alopecia	Hepatotoxicity
Late: Any time after completion of therapy			Secondary tumors or cancers

7.2 Adverse Event Characteristics

- **CTCAE term (AE description) and grade:** The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 4.0. A copy of the CTCAE version 4.0 can be downloaded from the CTEP web site http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

- **For expedited reporting purposes only:**
  AEs for the investigational agent that are bold and italicized in the CAEPR (i.e., those listed in the SPEER column, section 7.1.1) should be reported through CTEP-AERS only if the grade is above the grade provided in the SPEER.

- **Attribution** of the AE:
  - Definite – The AE *is clearly related* to the study treatment.
  - Probable – The AE *is likely related* to the study treatment.
  - Possible – The AE *may be related* to the study treatment.
  - Unlikely – The AE *is doubtfully related* to the study treatment.
  - Unrelated – The AE *is clearly NOT related* to the study treatment.

7.3 Expedited Adverse Event Reporting

7.3.1 Expedited AE reporting for this study must use CTEP-AERS (Adverse Event Reporting System), accessed via the CTEP Web site (http://ctep.cancer.gov). The reporting procedures to be followed are presented in the "NCI Guidelines for Investigators: Adverse Event Reporting Requirements for DCTD (CTEP and CIP) and DCP INDs and IDEs" which can be downloaded from the CTEP Web site (http://ctep.cancer.gov). These requirements are briefly outlined in the tables below (section 7.3.3).

In the rare occurrence when Internet connectivity is lost, a 24-hour notification is to be

made to CTEP by telephone at 301-897-7497. Once Internet connectivity is restored, the 24-hour notification phoned in must be entered electronically into CTEP-AERS by the original submitter at the site. Also, if internet connectivity is not re-established within 24 hours, notify the following by telephone and or email.

PBTC-033 Study Chair:
Patricia Baxter, M.D.
Pediatric Hematology-Oncology
Texas Children's Cancer Center
Baylor College of Medicine
1102 Bates Street Suite 1030 17
Houston Texas 77030
Tel: 832-824-4681
Fax: 832-825-4038
Email: pabaxter@txch.org

PBTC OBC:
Stacye Richardson, MSHS, BSMT
St. Jude Children's Research Hospital
Department of Biostatistics
Tel: (901) 595-3783
Fax: (901) 595-4173 or 595-4184
Email: stacye.richardson@stjude.org

7.3.2 CTEP-AERS is programmed for automatic electronic distribution of reports to the following individuals: Study Coordinator of the Lead Organization, Principal Investigator, and the local treating physician. CTEP-AERS provides a copy feature for other e-mail recipients.

7.3.3 Expedited Reporting Guidelines

Use the NCI protocol number and the protocol-specific patient ID assigned during trial registration on all reports.

Note: A death on study requires both routine and expedited reporting regardless of causality, unless as noted below. Attribution to treatment or other cause must be provided.

Death due to progressive disease should be reported as **Grade 5 "Neoplasms benign, malignant and unspecified (incl cysts and polyps) - Other (Progressive Disease)"** under the system organ class (SOC) of the same name. Evidence that the death was a manifestation of underlying disease (*e.g.*, radiological changes suggesting tumor growth or progression: clinical deterioration associated with a disease process) should be submitted.

Phase 1 and Early Phase 2 Studies: Expedited Reporting Requirements for Adverse Events that Occur on Studies under an IND/IDE within 30 Days of the Last Administration of the Investigational Agent/Intervention [1, 2]

FDA REPORTING REQUIREMENTS FOR SERIOUS ADVERSE EVENTS (21 CFR Part 312)
NOTE: Investigators **MUST** immediately report to the sponsor (NCI) **ANY** Serious Adverse Events, whether or not they are considered related to the investigational agent(s)/intervention (21 CFR 312.64)
An adverse event is considered serious if it results in **ANY** of the following outcomes:

1) Death
2) A life-threatening adverse event
3) An adverse event that results in inpatient hospitalization or prolongation of existing hospitalization for ≥ 24 hours
4) A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
5) A congenital anomaly/birth defect.
6) Important Medical Events (IME) that may not result in death, be life threatening, or require hospitalization may be considered serious when, based upon medical judgment, they may jeopardize the patient or

subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. (FDA, 21 CFR 312.32; ICH E2A and ICH E6).

ALL SERIOUS adverse events that meet the above criteria MUST be immediately reported to the NCI via CTEP-AERS within the timeframes detailed in the table below.

Hospitalization	Grade 1 and Grade 2 Timeframes	Grade 3-5 Timeframes
Resulting in Hospitalization ≥ 24 hrs	10 Calendar Days	24-Hour 5 Calendar Days
Not resulting in Hospitalization ≥ 24 hrs	Not required	

NOTE: Protocol specific exceptions to expedited reporting of serious adverse events are found in the Specific Protocol Exceptions to Expedited Reporting (SPEER) portion of the CAEPR.

Expedited AE reporting timelines are defined as:

- "24-Hour; 5 Calendar Days" - The AE must initially be reported via CTEP-AERS within 24 hours of learning of the AE, followed by a complete expedited report within 5 calendar days of the initial 24-hour report.
- "10 Calendar Days" - A complete expedited report on the AE must be submitted within 10 calendar days of learning of the AE.

[1]Serious adverse events that occur more than 30 days after the last administration of investigational agent/intervention and have an attribution of possible, probable, or definite require reporting as follows:
Expedited 24-hour notification followed by complete report within 5 calendar days for:

- All Grade 3, 4, and Grade 5 AEs

Expedited 10 calendar day reports for:

- Grade 2 AEs resulting in hospitalization or prolongation of hospitalization

[2]For studies using PET or SPECT IND agents, the AE reporting period is limited to 10 radioactive half-lives, rounded UP to the nearest whole day, after the agent/intervention was last administered. Footnote "1" above applies after this reporting period.

Effective Date: May 5, 2011

7.4 Routine Adverse Event Reporting

All Adverse Events **must** be reported in routine study data submissions. **AEs reported through CTEP-AERS must also be reported in routine study data submissions.**

7.5 Secondary Malignancy

A *secondary malignancy* is a cancer caused by treatment for a previous malignancy (*e.g.*, treatment with investigational agent/intervention, radiation or chemotherapy). A secondary malignancy is not considered a metastasis of the initial neoplasm.
CTEP requires all secondary malignancies that occur following treatment with an agent under an NCI IND/IDE be reported via CTEP-AERS. Three options are available to describe the event:

- Leukemia secondary to oncology chemotherapy (*e.g.*, acute myelocytic leukemia [45])
- Myelodysplastic syndrome (MDS)
- Treatment-related secondary malignancy

Any malignancy possibly related to cancer treatment (including AML/MDS) should also be reported via the routine reporting mechanisms outlined in each protocol.

7.6 Second Malignancy

A second malignancy is one unrelated to the treatment of a prior malignancy (and is **NOT** a metastasis from the initial malignancy). Second malignancies require **ONLY** routine reporting via CDUS unless otherwise specified.

8. PHARMACEUTICAL INFORMATION

A list of the adverse events and potential risks associated with the investigational or commercial agent administered in this study can be found in section 7.1.

8.1 ABT-888 (NSC # 737664)

Chemical Name: 1*H*-Benzimidazole-7-carboxamide, 2-[(2*R*)-2-methyl-2-pyrrolidinyl]-
Other Names: Veliparib, A-861695.0
Classification: Poly (ADP-ribosome) polymerase (PARP) Inhibitor
CAS Registry Number: 912444-00-9
Molecular Formula: $C_{13}H_{16}N_4O$ M.W.: 244.29

Mode of Action: ABT-888 is a potent oral PARP1/2 inhibitor whose pharmacological properties include high solubility (crosses the blood brain barrier), linear PK, and low potential drug-drug interactions. In vivo and in vitro, ABT-888 inhibits the formation of poly (ADP-ribose) (PAR) polymer and prevents the repair of DNA that is damaged by cytotoxic agents. Furthermore, ABT-888 can increase antitumor efficacy of temozolomide, cisplatin, carboplatin, cyclophosphamide, irinotecan, or radiation therapy.

Route of Administration: The oral solution can be administered with water or juice if needed. ABT-888 will be administered twice daily (~12 hours apart), Monday through Friday, during radiation therapy. The morning dose of ABT-888 should ideally be given 60-120 minutes prior to daily irradiation. For children who require sedation for irradiation and must remain NPO in the morning, ABT-888 may be given each night prior to bedtime, and the morning dose should be given shortly after the child recovers from sedation, adhering as closely to an every-12-hour schedule as possible. However, if permitted by the local sedation/anesthesia guidelines, ABT-888 may be given each morning prior to sedation, ideally 60-120 minutes prior to irradiation. See additional administration instructions in section 5.1. See **Appendix** D for calculated dose of ABT-888 capsules for patients taking capsules only.

During the maintenance therapy (starting on week 11 of protocol therapy), ABT-888 will be given twice daily (~12 hours apart), on days 1-5 of each 28-day course, with the morning dose of ABT-888 to be given 60-120 minutes prior to the morning temozolomide dose. The capsules should be swallowed whole without breakage. See Appendix E for the calculated dose for the ABT-888 capsules during maintenance for patients taking capsules only. ABT-888 capsules or the oral solution may be administered without regard to meals. If emesis occurs within 10 minutes of administration, the dose should be repeated.

Potential Drug Interactions: Clinical studies evaluating the metabolism of ABT-888 have not been conducted. However, results from the in vitro analysis reveal that this agent is metabolized by multiple isoenzymes – CYP1A1, 2D6, 2C19 and 3A4. ABT-888 is neither a potent inhibitor

nor a potent inducer of the CYP-450 isoenzymes. Use caution when concomitantly administer with drugs that are substrate, inhibitor, inducer of CYP1A1, 2D6, 2C19 and 3A4.

ABT-888 clears primarily in the urine as intact parent drug along with metabolites suggesting that renal function plays an important role in the drug clearance and its metabolites. Use caution when concomitantly administer with oxalipaltin, carboplatin, cisplatin, and topotecan in patients with pre-existing renal impairment.

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[redacted]

[redacted]

[redacted]

Availability: *ABT-888* in both capsule and oral solution formulations are provided to the NCI under a Collaborative Agreement between the Pharmaceutical Collaborator and the DCTD, NCI (see section 12.3).

8.1.2 Agent Ordering and Agent Accountability

NCI supplied agents may be requested by the Principal Investigator (or their authorized designee) at each participating institution. Pharmaceutical Management Branch (PMB) policy requires that agent be shipped directly to the institution where the patient is to be treated. PMB does not permit the transfer of agents between institutions (unless prior approval from PMB is obtained.) The CTEP assigned protocol number must be used for ordering all CTEP supplied investigational agents. The responsible investigator at each participating institution must be registered with CTEP, DCTD through an annual submission of FDA form 1572 (Statement of Investigator), Curriculum Vitae, Supplemental Investigator Data Form (IDF), and Financial Disclosure Form (FDF). If there are several participating investigators at one institution, CTEP supplied investigational agents for the study should be ordered under the name of one lead investigator at that institution.

Active CTEP-registered investigators and investigator-designated shipping designees and ordering designees can submit agent requests through the PMB Online Agent Order Processing (OAOP) application https://eapps-ctep.nci.nih.gov/OAOP/pages/login.jspx. Access to OAOP requires the establishment of a CTEP Identity and Access Management (IAM) account https://eapps-ctep.nci.nih.gov/iam/ and the maintenance of an "active" account status and a "current" password. For questions about drug orders, transfers, returns, or accountability, call (240) 276 - 6575 Monday through Friday between 8:30 am and 4:30 pm (ET) or email PMB after hours@mail.nih.gov anytime.

For the radiation phase of the protocol treatment, for patients taking capsules only, please order sufficient number of capsules for 30 days of treatment. For patients receiving the oral solution drug must be ordered and dispensed monthly during radiation.

Agent Inventory Records
The investigator, or a responsible party designated by the investigator, must maintain a careful record of the inventory and disposition of all agents received from DCTD using the NCI Drug Accountability Record Form (DARF). (See the NCI Investigator's Handbook for Procedures for Drug Accountability and Storage.)

8.2 Temozolomide (Temodar™; NSC#: 362856)

Sites should refer to the FDA approved package insert for additional information on the commercial drug.

Formulation:
Temozolomide is available in 5 mg, 20 mg, 100 mg, 140 mg, 180mg and 250 mg capsules.

Stability & Storage: Capsules should be stored at 25ºC (77ºF); excursions permitted to 15-30ºC (59-86ºF). The commercial product has an expiration date on the bottle.

Procedure for Proper Handling and Disposal
Chemotherapy precautions should be utilized in preparation.

Route/Frequency of Administration: Dose should be rounded to the nearest 5 mg. See Appendix E for dosing determination. Temozolomide should be administered 60-120 minutes after the morning ABT-888 dose. If a patient has emesis within 10 minutes of receiving a dose, the dose should be repeated.

For patients unable to swallow intact capsules, capsules may be opened and mixed with apple juice or applesauce immediately prior to administration. If opening of capsules is necessary, it should be performed by hospital staff trained in handling chemotherapeutics whenever possible. However, if necessary, adult family members are allowed to open the temozolomide capsules at home, provided that they follow the instructions in Appendix C strictly, as well any additional instructions given by hospital pharmacists trained in handling chemotherapeutics.

Agent Ordering
Temozolomide is available from commercial sources by the treating hospital pharmacy.

9. CORRELATIVE AND SPECIAL STUDIES

9.1 Laboratory Correlative Studies

9.1.1 Plasma Pharmacokinetics Studies

Participation in the pharmacokinetic studies is required during the Phase I portion of the study and strongly encouraged but optional during Phase II. Plasma pharmacokinetics samples will be obtained on days 1 (first dose) and 4 (steady state) during the first week of ABT-888 and

radiation treatment. If day 4 falls on a Saturday, please collect steady state PK samples on day 3. If day 4 falls on a Sunday, please collect steady state PK samples on day 5.

9.1.1.1 *Sampling Strategy for Pharmacokinetic Study*

First dose pharmacokinetic study of ABT-888, Day 1 of ABT-888 and Radiation treatment
Blood samples will be collected prior to the first dose of ABT-888 on day 1 of radiation treatment, and then at 0.5, 1, 2 and 6-8 hours after the first dose of ABT-888. Blood samples may be obtained either by repeated phlebotomies or through temporary or permanent intravenous catheters/devices. The pre-ABT-888 sample may be obtained at study entry, provided that the specimen is processed and stored appropriately per section 9.1.1.2.

For children who require sedation for radiation and are not able to take the p.o. medication due to local requirements for NPO prior to sedation, the pre-ABT-888 sample may be obtained at study entry or at any time prior to the first dose of ABT-888 (to be administered the night prior to first day of radiation treatment). Subsequent PK samples outlined above (at 0.5, 1, 2 and 6-8 hours after a dose of ABT-888) should be collected after the child awakens from sedation and after the second dose of ABT-888 is given on day 1 of radiation treatment.

Steady state pharmacokinetic study of ABT-888, Day 4 of ABT-888 and Radiation treatment
Blood samples should be obtained prior to the A.M. ABT-888 dose on day 4 of radiation treatment, and then at 2 hours after the A.M. dose. Blood samples may be obtained either by repeated phlebotomies or through temporary or permanent intravenous catheters/devices.

For children who require sedation for radiation and must remain NPO in the morning, the day 4 samples should be obtained after the child awakens from sedation on day 4 and then at 2 hours after the day-4 ABT-888 dose has been given.

9.1.1.2 *Sampling Collection and Processing Instructions*

Blood samples (a minimum of 2.5 ml for children < 12 kg, and 3-5 ml for children > 12 kg) should only be collected in potassium EDTA tubes, provided by AbbVie. All information about the PK kits and ordering instructions are available on the PBTC-033 members' website in the Protocol Specific Instructions/Forms section. Sites are to record the exact time that the sample is drawn along with the exact time that the study drug is administered on the PK Sample Transmittal form located in the PBTC-033 Protocol Specific Instructions/Forms section of the PBTC members' webpage.

9.1.1.3 *Sampling Handling and Shipping Instructions*

Samples should be immediately inverted 8 to 10 times to reduce the likelihood of clot formation and centrifuged within 30 minutes at sufficient speed and time to separate plasma (e.g. 1300 x g for 10 minutes). Plasma should be transferred to labeled cryovials (labels and cryovials provided by AbbVie) within one hour of collection and stored immediately at -20°C or colder.

Each tube must be labeled with the labels provided in the collection kits. These will include the patient's accession number, the study number, and the planned time of sampling relative to dosing. Data should be recorded on the Pharmacokinetic Sample Transmittal Form located on the PBTC-033 'Protocol Specific Instructions/Forms' section of the PBTC Members' webpage.

The transmittal form must accompany the sample(s).

Samples from day 1 and day 4 PK studies for each patient should be batched together and shipped via express carrier (Monday through Wednesday only). Samples are to be shipped at -20°C or colder (on dry ice) to:

[redacted]

Please ship each patient's PK samples as soon as the complete set has been collected rather than waiting to batch multiple patients' samples together. Please FAX the completed form to AbbVie Sample Receiving on the day of shipment. Also, be sure to include the completed PK Specimen transmittal form in the shipment as it will provide an inventory of the samples included in the shipment. PK samples must be shipped within 30 days of Day 4 sample collection in order to receive cost reimbursement.

9.1.1.4 *Description of Pharmacokinetic Analysis*
Plasma concentration of ABT-888 will be analyzed using a previously validated LC/MS/MS technique. These samples will be analyzed at AbbVie Laboratories, located in AbbVie Park, IL.

9.1.2 Correlative Biology Studies in PBMC
Participation in the correlative biology studies is **strongly encouraged but optional**.

9.1.2.1 *Collection of Specimen(s)*
Whole blood samples (a minimum of 5 ml for children < 12 kg, and 10 ml for children > 12 kg) should be collected in either ACDA (Acid citrate dextrose solution A; yellow top) or heparinized (sodium or lithium heparin; green top) tubes. Blood samples should be collected at the following time points:

1. Prior to start of first dose of ABT-888 and radiation treatment (sample may be collected at the time of study entry or any time prior to start of 1st dose of ABT-888)
2. On day 3-4 during the third week of ABT-888 and radiation treatment, 2 hours after the day-time dose of ABT-888; if day 4 falls on a Friday, collect this sample on day 3
3. On day 3-4 during the sixth week of ABT-888 and radiation treatment, 2 hours after the day-time dose of ABT-888; if day 4 falls on a Friday, collect this sample on day 3
4. On day 4 during the first course of maintenance ABT-888 and TMZ, 2 hours after the day-time dose of ABT-888; if day 4 falls on a Friday, collect this sample on day 3; if day 4 falls on a Sunday, collect this sample on day 5; if day 4 falls on a Saturday, collect this sample on day 2.

Record the exact date and time that the sample is drawn along with the exact time ABT-888 was administered on the Biologic Specimen Transmittal Form located on the PBTC-033 Protocol Specific Instructions/Forms section of the PBTC members' webpage.

If possible, avoid collecting samples on a Friday to avoid weekend sample shipment. If unavoidable, please contact Drs. Patricia Baxter at (832) 824-4681 (pabaxter@txch.org) or Jack Su at (832) 822-4306 (jmsu@txch.org) to arrange for Saturday delivery.

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

9.1.2.3 *Sample Labeling*
Each tube must be labeled with the patient's accession number, the study number, the exact date and time the sample was drawn and the exact time that the drug was administered. Data should be recorded on the Biologic Specimen Transmittal Form located on the PBTC-033 Protocol Specific Instructions/Forms section of the PBTC members' webpage. The transmittal form must accompany the sample(s).

9.1.2.4 *Shipping Instructions*
Each sample should be shipped immediately on the day of collection. Samples should be stored at room temperature until shipment. Sample handling and shipping instructions are available on the PBTC-033 Protocol Specific Instructions/Forms section of the PBTC members' webpage. Samples should be shipped by priority overnight via express carrier (Monday through Thursday only).

Patricia Baxter, MD; Attention: Elizabeth Hinojosa
Feigin Center
1102 Bates St., Room 1030
Baylor College of Medicine
Houston, TX 77030
(832) 824-4688

Prior to shipping, please contact Drs. Patricia Baxter at (832) 824-4681(pabaxter@txch.org) or Jack Su at (832) 822-4306 (jmsu@txch.org) for notification of sample shipment.

9.1.2.5 *Sample Analysis*
PBMCs will be isolated using a CPT™ Cell Preparation Tube with Sodium Citrate. PARP activity will be measured using an established ELISA assay for PAR formation by AbbVie Laboratories.[46] NHEJ activity will be analyzed via a published assay.[47] PBMC γ-H2AX levels will be determined using flow cytometry and a monoclonal antibody against γ-H2AX conjugated with GFP. PBMC NHEJ activity and γ-H2AX levels will be analyzed in the laboratory of Dr. Xiao-Nan Li, located at Baylor College of Medicine, Houston, TX.

9.1.3 Correlative Biology Studies in Tumors
Participation in correlative studies for tumor specimens is optional but strongly encouraged.

Frozen Tumor

9.1.3.1 *Collection and Processing*

If biopsied tumor is available, the sample should be frozen in liquid nitrogen as soon as possible and shipped on dry ice. Data should be recorded on the Biologic Specimen Transmittal Form located on the PBTC 033 Protocol Specific Instructions/Forms section of the PBTC members' webpage.

9.1.3.2 *Labeling and Shipping*

Samples should be labeled with the patient's PBTC accession number and the date of surgery. Frozen tumor specimens should be shipped Monday through Thursday only. The specimen should be accompanied by the Correlative Biology Study Form and shipped by overnight carrier to:

Patricia Baxter, MD; Attention: Elizabeth Hinojosa
Feigin Center
1102 Bates St., Room 1030
Baylor College of Medicine
Houston, TX 77030
(832) 824-4688

Prior to shipping, please contact Drs. Patricia Baxter at (832) 824-4681(pabaxter@txch.org) or Jack Su at (832) 822-4306 (jmsu@txch.org) for notification of sample shipment.

9.1.3.3 *Sample Analysis*

PARP activity will be measured using an established ELISA assay for PAR formation by AbbVie Laboratories.[46] NHEJ activity will be evaluated by an enzymatic assay,[47] and protein levels of Ku 70, Ku 80, DNA-PK, BRCA 1, BRCA 2, Rad51, and ATM will be measured by Western analysis in Dr. Xiao-Nan Li's laboratory.

FFPE Slides

9.1.3.4 *Collection and Processing*

Fifteen to twenty unstained PLUS glass slides, containing 5-micron thick tumor sections from FFPE tumor blocks will ALSO be requested in the event that frozen tumor specimens submitted are of insufficient amount or inadequate quality.

9.1.3.5 *Labeling and Shipping*

Slides should be labeled with the patient's PBTC accession number and the date of surgery. The Correlative Biology Study Form located on the PBTC-033 Protocol Specific Instructions/Forms section of the PBTC members' webpage should be completed and shipped with the specimens. Slides should be shipped Monday through Thursday only. The slides should be shipped at room temperature to the address listed in 8.3.2. Prior to shipping, please contact Drs. Patricia Baxter at (832) 824-4681(pabaxter@txch.org) or Jack Su at (832) 822-4306 (jmsu@txch.org) for notification of shipment.

9.1.3.6 *Sample Analysis*
Immunohistochemical staining of PARP, Ku 70, Ku 80, DNA-PK, BRCA 1, BRCA 2, Rad51, and ATM will be performed by standard techniques in Dr. Adekunle Adesina's laboratory at Baylor College of Medicine, Houston, Texas.

9.1.4 Urine Biomarkers
Participation in the urine biomarker study is strongly encouraged but optional.

9.1.4.1 *Sampling Strategy*
Urine will be collected at time of enrollment (pre-treatment), post ABT-888 plus radiation then at the time of each scheduled MRI. Urine will be collected throughout the time that each patient is receiving protocol treatment until disease progression.

9.1.4.2 *Sample Collection*
10-30 milliliters of urine will be collected in a specimen cup and immediately placed in ice. The specimen can remain on ice in the original container for 1-2 hours until transfer to a freezer for storage (-20 Celsius). Samples should be maintained at -20 C until shipped. Urine can be collected as a mid-stream catch while voiding or can be collected from a urimeter if the child is catheterized. It is necessary to keep the urine refrigerated to prevent the degradation of peptides. The MMPs are particularly sensitive to warmer temperatures and other enzymes within the urine will break down biomarkers if not inactivated by refrigeration.

9.1.4.3 *Sample Labeling*
Each urine specimen must be labeled with the patient's accession number, the study number (PBTC-033) and the date collected. Data should be recorded on the Urine Specimen Transmittal Form located on the PBTC-033 Protocol Specific Instructions/Forms section of the PBTC members' webpage. The transmittal form must accompany the sample(s).

9.1.4.4 *Shipping Instructions*
Samples must be shipped monthly from the site to the laboratory to receive cost reimbursement. The sample collection and shipping dates must be documented in the eCRF. All samples should be forwarded via FedEx, Monday through Thursday, by completing the internet form at http://www.fedex.com/us/ and requesting FedEx to email the laboratory. Fed Ex account information for the shipment of samples can be obtained on the PBTC website. Weekend deliveries are not permitted. All urine specimens along with a completed Urine Specimen Transmittal Form should be placed within a Styrofoam shipping package with dry ice and mailed to the attention of Micah Duggins-Warf.

Micah Duggins-Warf
Children's Hospital Boston
Karp Research Building, 12th Floor
1 Blackfan Circle
Boston MA 02115
(617) 919-2211
Email: micah.duggins-warf@childrens.harvard.edu

9.1.4.5 *Description of Urine Biomarker Assay*

All samples will be analyzed for the presence or absence of each of the 8 biomarkers. Zymography will be performed to analyze for MMP-2, MMP-9 and MMP-9/NGAL in a blinded fashion by the laboratory examiner and recorded as a positive or negative result, following a protocol well-established in our laboratory which has been validated in the literature.39

Zymography

Samples will be frozen immediately after collection and stored frozen (-20C) until assay. Aliquots of each sample will be centrifuged at 4,000 rpm for 5 min at 4C and the supernatants collected. Urine samples (30 uL) will be mixed with buffer consisting of 4% SDS, 0.15 mol/L Tris (pH 6.8), 20% (v/v) glycerol, and 0.5% (w/v) brom-phenol blue. Samples will be applied, without boiling, into wells of a 4% acrylamide Laemmli stacking gel/10% SDS-acrylamide separating gel containing 0.1% (w/v) gelatin (Life Technologies, Inc.) on a mini gel apparatus. Gels will be run at 15 mA/gel during stacking and at 20 mA/gel during the resolving phase at room temperature. After electrophoresis, the gels will be soaked in 2.5% Triton X-100 with gentle shaking for 30 min at ambient temperature with one change of detergent solution. The gels will then be rinsed and incubated overnight at 37ºC in substrate buffer [50 mmol/L Tris-HCI buffer (pH 8), 5 mmol/L CaCl2, and 0.02% NaN3,]. After incubation, gels will be stained for 15 to 30 min in 0.5% Coomassie Blue R-250 in acetic acid, isopropyl alcohol, and water (1:3:6); destained in acetic acid, ethanol, and water (1:3:6), and photographed. The presence or absence of each species (MMP-2, MMP-9, MMP-9/NGAL) will be recorded and relative intensities (if present) noted.

ELISA

ELISA will be used to establish quantitative levels of each of the 8 biomarkers. Kits will be obtained from the manufacturers (R&D systems and Bioplex/Bio-Rad). Specimens, standards and reagents will be prepared according to manufacturer's instructions. Protein concentration will be determined via the Bradford method using bovine serum albumin as the standard. Results will be recorded as ng/ml or pg/L.

9.2 Special Studies

9.2.1 Neuroimaging Studies

Standard MRI will be obtained within 2 weeks prior to registration, at the end of radiation therapy (week 10), within 1 week prior to courses 3, 5, and 8 of maintenance and at the end of treatment. MR perfusion and permeability studies and DTI will also be obtained at the same time points as standard MRI through week 26 (6 months from initiation of treatment).

For patients showing possible tumor progression on MRI during the first 6 months after the initiation of ABT-888 and irradiation (on scheduled MRI studies), the treating physician will have the option of allowing the patient to remain on study, continuing protocol therapy, and repeating disease reassessment in 4-6 weeks. The investigational sequences should be obtained on this additional scan which is completed due to possible pseudoprogression. 3T imaging is the preferred modality of choice.

Standard MR imaging will include Sagittal T1 MPRAGE, axial FLAIR, axial T2,

postgadolinium sagittal T1 MPRAGE (with reconstructions) and axial T2 images. Protocol standard parameters will be distributed to the sites and posted on the PBTC NIC website.

Volumetric analyses will be done at the Neuroimaging Center (NIC, Children's Hospital Boston) via the Vitrea (Vitrea™) workstation, from the axial FLAIR and T1-weighted post-contrast brain images.

9.2.2 MRI Permeability and Perfusion Imaging

The perfusion protocol will be performed using T1-weighted dynamic contrast-enhanced (DCE) permeability MRI to assess immediate biological activity followed by T2*-weighted dynamic susceptibility contrast (DSC) perfusion MRI technique. DSC perfusion MRI dynamics will allow assessment of the hemodynamic parameter relative cerebral blood volume (rCBV). DCE permeability MRI metrics will include the volume transfer constant between plasma and extravascular extracellular space (K^{trans}), fractional blood-plasma volume (V_p), and the volume of the extravascular extracellular space per unit volume tissue (V_e). Both DCE and DSC MRI-derived data will be complementary to conventional contrast-enhanced MR imaging.

9.2.2.1 *DCE permeability MRI*

Please note that there will be a total of 5 sequences: 4 for T1 mapping and the DCE with injection). **Also, it is important that the same patient MUST be scanned on the same exact scanner (not just the brand/tesla, but actual scanner) if more than one exam is to be done.** A 3D (not 2D) fast gradient echo type of sequences (fast SPGR, FLASH, THRIVE) must be used. This will be performed as 3D slab in the axial plane. Normalization or intensity correction or flow correction filters such as CLEAR, SCIC or PURE must not be used for any of the series. The slice locations and positioning for the T_1 mapping and the dynamic series *MUST* be identical (same matrix, slices, FOV, TR, TE, except NEX and FA). Hence copying of the slices is needed. The TR and TE for all 4 series (4 T_1 maps plus a dynamic series) should be identical. For GE systems, reduce Turbo Factor to 1 or 0 if TR and TE do not match across series. T_1 Maps should be acquired with 2 signal averages and the Dynamic Series with 1. Temporal Resolution of "T1 DCE" series (scan time per phase/measurement) *should be less than or equal to 6 Seconds, with NO gaps between phases.* ASSET/IPAT/Parallel Imaging Parallel imaging is set to be OFF, however, if it is not possible to achieve a temporal resolution of less than 6 seconds, this should be set to a factor of 2. The dynamic series should last *5 minutes* in total scan time (*excluding* T1 mapping series).

The table below describes the image acquisition parameters for the T_1 map sequences as well as the dynamic scan, ***in the order of acquisition (first T1 maps then T1 DCE)***. Make sure this happens ***before*** DSC perfusion MRI.

The first half dose of contrast agent to be administered 20 sec into "T1 DCE" sequence. Do NOT inject prior to T1 DCE or during T1 maps (see **Table 16** and **Table 17** below).

Table 16

"T1 DCE"			
Series Name	**Sequence**	**Flip Angle**	**Notes**
T1 map15	3D fast GRE	15 degrees	Axial, 2 NEX
T1 map10	3D fast GRE	10 degrees	Axial, 2 NEX
T1 map05	3D fast GRE	5 degrees	Axial, 2 NEX
T1 map02	3D fast GRE	2 degrees	Axial, 2 NEX
T1 DCE	Dynamic Series, 3D fast GRE	15 degrees	Axial, 1 NEX, inject 20 sec into this

Table 17

3D T1W specs for T_1 Maps and Dynamic Series	
Sequence type	Spoiled gradient echo
Imaging mode	**3D**
Slice orientation	Axial
Frequency direction	A/P
Phase direction	R/L
FOV - frequency	220 mm
FOV - phase	220 mm
Matrix - frequency	256
Matrix - phase	160-192
In-plane resolution	≤ 1 mm
Fat-suppression	No Fat Sat
TR	~4 msec
TE	Less than 2 ms or min full
TI (STIR sequence)	N/A
Flip Angle	DCE -15 degrees; T1 maps - 2, 5, 10 and 15
Slice thickness (acquired, not interpolated)	5mm, maximum 6mm
Number of slices	Minimum 10 prior to zero fill
Slice Gap	No gap
Parallel imaging factor	≤ 2
Number of averages	1 for DCE, 2 for T1 maps
k-space ordering	standard, non-centric
Temporal Resolution of "T1 DCE": (seconds per phase/measurement)	≤ 6 seconds
"T1 DCE" imaging duration	≥ 5 minutes

Run the Dynamic multi-phase "T1 DCE" at flip angle of 15 degrees – enable multi-phase (on GE systems) and increase the number of phases (or measurements) until the scan time is **six** minutes. Contrast injection should be delivered at 20 sec into T1 DCE, not earlier. Injection rate is 2 ml/second at 0.05 mmol/kg body weight followed by a 10 cc saline flush at the same rate **(all should use the same type of contrast agent).**

9.2.2.2 *Diffusion tensor imaging*

Diffusion tensor imaging (DTI) or axial T2 weighted imaging can be performed between the DCE and DSC MRI acquisitions. In addition to providing permeability metrics, the gadolinium contrast agent from the DCE acquisition will also serve as a "preload" to help correct for leakage effects for the DSC perfusion acquisition. [20]

9.2.2.3 *DSC perfusion MRI*

An axial 2D T2* GRE-EPI sequence will be used. TR = 2000 ms, TE = 23 ms, matrix = 128 x 128, FOV = 240 mm, frequency direction R-L, slice thickness = 5.0 mm with 2 mm gap, flip angle = 60 degrees, NEX = 1. Repeat 50-60 times. Total acquisition time ~ 2 minutes. Begin bolus injection (2 ml/sec) of 0.05mmol/kg BW GdDTPA at 20secs after scanning starts followed by a 10 cc saline flush at the same rate. Regional rates of transverse relaxation enhancement ($\Delta R2^*$) during contrast agent passage will be calculated from: $\Delta R2^*(t) = (-1/TE)\ \text{In}\ [S(t)/S(0)]$ from which estimates of rCBV will be derived.

Perfusion MR images will be transferred to the GE Advantage Workstation (4.3.05); Functool (4.4.05) (GE Healthcare, Milwaukee, WI) to calculate rCBV maps on a voxel-wise basis. User defined regions of interest (ROIs) measuring 3-5 mm will be placed within areas that demonstrate maximal CBV based on color-overlay maps. Effort will be made to exclude large vessels and necrotic-appearing regions from the ROIs. An ROI will also be placed in the frontal normal appearing white matter (NAWM) to provide relative CBV (rCBV) values to normalize perfusion data and enable comparison between patient and between serial studies.[18]

Permeability MR Images will be transferred to the iCAD Workstation (iCAD, Inc., Nashua, NH). This utilizes a 2 compartmental pharmacokinetic model allowing voxel-wise calculation of K^{trans}, V_p, and V_e. User defined ROIs encompassing the entire contrast-enhancing lesion as well as multiple smaller 3-5 mm ROIs will be placed within areas that demonstrate maximal K^{trans}, V_p, and V_e based on color-overlay maps. Effort will be made to exclude large vessels and necrotic-appearing regions from the ROIs.

9.2.3 MR Diffusion Imaging

MR Diffusion Imaging will consist of 35 direction DTI sequences: slice thickness 2.2 mm, TR= 8800 ms, TE= 88ms, FOV 220 mm, b value= 1000 s/mm^2, 35 directions. Full protocol will be provided on the PBTC NIC website.

9.2.4 Image Transfer

Standard MRI, perfusion/ permeability and DTI images will be electronically transferred to the PBTC Neuroimaging Center (NIC). All patient specific data are stripped from the images and replaced with PBTC case-ids prior to sending images to the NIC. All image data transfer is accomplished using PGP (pretty-good-privacy) 128-bit encryption which meets industry standard for secure communication.

9.2.5 Central Review

Central review of imaging studies will be conducted through the PBTC Neuroimaging Center (NIC). The director and one neuroradiologist at NIC will review the imaging studies for response and pseudo-progression at study completion. Post processing of perfusion/permeability studies will be done at USC.

10. STUDY CALENDAR

Table 18

Standard Clinical and Laboratory Assessments – Phase I								
	Pre-therapy	ABT888 and Radiation (Course 1.1)			Maintenance (Courses 2.1 – 2.23)			End of Treatment
PHYSICAL ASSESSMENTS		ABT 888 + RT	Rest Period	Week 10 – 11[10]	Weekly Courses 1-10	Prior to Courses 2-10	Extended Therapy Courses 11-23	
Medical history, physical exam and diary review	X	Weekly	X[9]	X		X	X[12]	X
Vital signs	X	Weekly	X[9]	X		X	X[12]	X
Performance status	X	Weekly	X[9]	X		X	X[12]	X
Neurologic exam	X		X[9]	X		X	X[12]	X
Height, Weight, BSA	X		X[9]	X		X	X[12]	
LABORATORY EVALUATIONS								
CBC (WBC, Hgb, Hct, Platelets, ANC, ALC)	X	Weekly[1]	X[9]	X	X[1]		X[13]	X
Serum Chemistry (Electrolytes, BUN, Creatinine, Calcium, Magnesium, Phosphorous, SGOT(AST), SGPT(ALT), Total Bilirubin, Albumin)	X	Weekly[2]	X[9]	X		X[2]	X[13]	X
Pregnancy test [3]	X			X		X	X	
IMAGING ASSESSMENTS								
Brain MRI	X[4]			X[4]		X[4,11]	X[14]	X[11]
MRI Perfusion/Permeability	X[4]			X[4]		X [4,11]		X[11]
Diffusion Tensor Imaging (DTI)	X[4]			X[4]		X [4,11]		X[11]
Correlative Studies								
Pharmacokinetic Studies[5]	X	X						
Biology Study (PARP,NHEJ and γH2AX) in PBMC's[6]	X	X			X[6]			
Biology study – pre-trial tumor material	X[7]							
Urine Biomarker[8]	X			X		X[8]		

1. CBC is done weekly for all courses. Patients with dose limiting hematologic toxicity (≥Grade 3 thrombocytopenia or Grade 4 neutropenia) should have CBC at least twice weekly (3-4 days apart) until toxicities improve to grade 2 or less
2. Patients with grade 3 or 4 non-hematologic toxicity should have relevant laboratory tests repeated twice weekly (3-4 days apart) until they recover to grade 2 or less
3. Serum or urine pregnancy test is to be repeated within 72 hours prior to enrollment **in females of childbearing potential** and prior to each course during maintenance
4. Neuroimaging assessments are to be completed within 2 weeks of registration, at the end of radiation + ABT888, (week 10-11), within 1 week prior to courses 3, 5, 8 and at the end of maintenance. See footnote #11 for details of investigational imaging procedures. For patients with possible tumor progression on week 10, 18, or 26 imaging studies, see guidelines in section 2.4.2 or 11.1.4 for frequency of next imaging study.
5. Participation in the pharmacokinetic study is **required for the Phase I portion of the study.** See section 9.1.1 for collection schedule
6. Participation in the correlative biology study in PBMC's is **optional.** See section 9.1.2 for collection schedule
7. Participation the biology study – pre-trial tumor material is optional. See section 9.1.3 for processing and shipping instructions.
8. Participation in the urine correlative study is **optional**. Urine samples should be collected at the time of each scheduled MRI (section 9.1.4)
9. During the rest period, each patient should have at least one visit between weeks 7 and 10.
10. Laboratory tests and assessments may be completed ±1 week of week 10, prior to starting Maintenance.
11. For patients showing possible tumor progression on MRI during the first 6 months after initiation of ABT-888 and radiation (on required MRI studies performed at week 10, week 18, or 26), the treating physician will have the option of allowing the patient to remain on study, continuing protocol therapy, and repeating disease reassessment in 4-6 weeks. The repeat MRI should include MRI perfusion/permeability and DTI. ***MRI Perfusion/Permeability and DTI will only be obtained on routine MRI studies through week 26; the investigational sequences should be obtained on a scan 4-6 weeks after the week 26 study IF the patient is being handled as a potential pseudoprogression.***
12. If a patient receives more than 10 courses, evaluations in subsequent courses should be obtained at the discretion of the treating physician
13. It is recommended that labs be obtained at least every two weeks during the extended treatment period (i.e. beyond course 10).
14. It is recommended that standard MRI scans should be done at least every three months during the extended treatment period (i.e. beyond course 10). Additional studies may be done as clinically indicated at the investigator's discretion.

Note: Obtain other studies as clinically indicated

Pre-therapy laboratory evaluations within 7 days of starting treatment will be considered Day 1, Course 1 evaluations

Table 19

Standard Clinical and Laboratory Assessments – Phase II								
	Pre-therapy	ABT888 and Radiation (Course 1.1)			Maintenance (Courses 2.1-2.23)			End of Treatment
PHYSICAL ASSESSMENTS		ABT 888 + RT	Rest Period	Week 10 – 11[10]	Weekly Courses 1-10	Prior to Courses 2-10	Extended Therapy Courses 11-23	
Medical history, physical exam and diary review	X	Weekly	X[9]	X		X	X[12]	X
Vital signs	X	Weekly	X[9]	X		X	X[12]	X
Performance status	X	Weekly	X[9]	X		X	X[12]	X
Neurologic exam	X		X[9]	X		X	X[12]	X
Height, Weight, BSA	X		X[9]	X		X	X[12]	
LABORATORY EVALUATIONS								
CBC (WBC, Hgb, Hct, Platelets, ANC, ALC)	X	Weekly[1]	X[9]	X	X[1]		X[13]	X
Serum Chemistry (Electrolytes, BUN, Creatinine, SGOT(AST), SGPT(ALT), Total Bilirubin,)	X	Weekly[2]	X[9]	X		X[2]	X[13]	X
Pregnancy test [3]	X			X		X	X	
IMAGING ASSESSMENTS								
Brain MRI	X[4]			X[4]		X [4,11]	X[14]	X[11]
MRI Perfusion/Permeability	X[4]			X[4]		X [4,11]		X[11]
Diffusion Tensor Imaging (DTI)	X[4]			X[4]		X [4,11]		X[11]
Correlative Studies								
Pharmacokinetic Studies[5]	X	X						
Biology Study (PARP,NHEJ and γH2AX) in PBMC's[6]	X	X			X[6]			
Biology study – Pre-trial tumor material[7]	X							
Urine Biomarker[8]	X			X		X[8]		

1. CBC is done weekly for all courses. Patients with dose limiting hematologic toxicity (≥Grade 3 thrombocytopenia or Grade 4 neutropenia) should have CBC at least twice weekly (3-4 days apart) until toxicities improve to grade 2 or less
2. Patients with grade 3 or 4 non-hematologic toxicity should have relevant laboratory tests repeated twice weekly (3-4 days apart) until they recover to grade 2 or less
3. Serum or urine pregnancy test is to be repeated within 72 hours prior to enrollment **in females of childbearing potential** and prior to each course during maintenance
4. Neuroimaging assessments are to be completed within 2 weeks of registration, at the end of radiation + ABT888, (week 10-11), within 1 week prior to courses 3, 5, 8 and at the end of maintenance. See footnote #11 for details of investigational imaging procedures. For patients with possible tumor progression on week 10, 18, or 26 imaging studies, see guidelines in section 2.4.2 or 11.1.4 for frequency of next imaging study.
5. Participation in the pharmacokinetic study is **optional for the Phase II portion of the study.** See section 9.1.1 for collection schedule
6. Participation in the correlative biology study in PBMC's is **optional.** See section 9.1.2 9.1.2for collection schedule
7. Participation in the correlative biology study pre-trial tumor material is optional. See Section 9.1.3 for processing and shipping instructions
8. Participation in the urine correlative study is **optional**. Urine samples should be collected at the time of each scheduled MR (section 9.1.4)
9. During the rest period, each patient should have at least one visit between weeks 7 and 10.
10. Laboratory tests and assessments may be completed ±1 week of week 10, prior to starting Maintenance.
11. For patients showing possible tumor progression on MRI during the first 6 months after initiation of ABT-888 and radiation (on required MRI studies performed at week 10, week 18, or 26), the treating physician will have the option of allowing the patient to remain on study, continuing protocol therapy, and repeating disease reassessment in 4-6 weeks. The repeat MRI should include MRI perfusion/permeability and DTI. ***MRI Perfusion/Permeability and DTI will only be obtained on routine MRI studies through week 26; the investigational sequences should be obtained on a scan 4-6 weeks after the week 26 study IF the patient is being handled as a potential pseudoprogression.***
12. If a patient receives more than 10 courses, evaluations in subsequent courses should be obtained at the discretion of the treating physician
13. It is recommended that labs be obtained at least every two weeks during the extended treatment period (i.e. beyond course 10).
14. It is recommended that standard MRI scans should be done at least every three months during the extended treatment period (i.e. beyond course 10). Additional studies may be done as clinically indicated at the investigator's discretion.

Note: Obtain other studies as clinically indicated

Pre-therapy laboratory evaluations within 7 days of starting treatment will be considered Day 1, Course 1 evaluations

11. MEASUREMENT OF EFFECT

11.1 Tumor Response Criteria

11.1.1 Complete Response (CR)

Complete disappearance on MR/CT of all enhancing tumor and mass effect, on a stable or decreasing dose of corticosteroids (or receiving only adrenal replacement doses), accompanied by a stable or improving neurologic examination, and maintained for at least 8 weeks.

11.1.2 Partial Response (PR)

Greater than or equal to 50% reduction in tumor size by bi-dimensional measurement, as compared with the baseline measurements, on a stable or decreasing dose of corticosteroids, accompanied by a stable or improving neurologic examination, and maintained for at least 8 weeks

11.1.3 Stable Disease (SD)

Neurologic exam is at least stable and maintenance corticosteroid dose not increased, and MR/CT imaging meets neither the criteria for PR nor the criteria for Progressive Disease. If this category is to be reported as of possible clinical benefit, Stable Disease status must be maintained for 16 weeks.

11.1.4 Progressive Disease (PD)

Progressive Disease (PD): Progressive neurologic abnormalities or worsening neurologic status not explained by causes unrelated to tumor progression (e.g., anticonvulsant or corticosteroid toxicity, electrolyte disturbances, sepsis, hyperglycemia, etc.), OR a greater than 25% increase in the bi-dimensional measurement, taking as a reference the smallest disease measurement recorded since the start of protocol therapy, OR the appearance of a new lesion, OR increasing doses of corticosteroids required to maintain stable neurological status or imaging.

* Consideration for possible pseudo-progression

For patients showing possible radiographic evidence of tumor progression on MRI during the first 6 months after initiation of ABT-888 and radiation (on required MRI studies performed at week 10, week 18, or 26), the treating physician will have the option of allowing the patient to remain on study, continuing protocol therapy, and repeating disease reassessment in 4-6 weeks. Provided that the patient does not show clinical deterioration consistent with tumor progression, has been on a stable or declining dose of steroids, and the subsequent MRI demonstrates tumor regression or stable disease, then the patient will remain on study and continue protocol therapy, and the frequency of subsequent MRI will revert to pre-specified intervals. If the repeat MRI after 4-6 weeks shows disease progression, rather than pseudo-progression, then the time of progression will be the date of the initial MRI, not the follow up scan. The addition of steroids is allowed, at the time of suspected pseudo-progression, as long as the patient can be maintained on a decreasing dose.

12. DATA REPORTING / REGULATORY REQUIREMENTS

Adverse event lists, guidelines, and instructions for AE reporting can be found in section 7 (Adverse Events: List and Reporting Requirements).

12.1 Data Reporting

12.1.1 Responsibility for Data Submission

Pre-treatment, on-study and off-treatment data, as well as patient response data are to be recorded in the electronic data collection screens using the PBTC RDC database. See the Required Data and Timetable for Submission form located on the 033 Protocol webpage for the schedule. For assistance, contact the PBTC Protocol Coordinator, Stacye Richardson at (901) 595-3783, email: stacye.richardson@stjude.org. An optional roadmap is located on the PBTC-033 Protocol Webpage.

12.1.2 Method

This study will be monitored by the Clinical Data Update System (CDUS) Version 3.0. Cumulative protocol- and patient-specific CDUS data will be submitted electronically to CTEP on a quarterly basis, either by FTP burst of data or via the CDS web application. Reports are due January 31, April 30, July 31, and October 31. Instructions for submitting data using the CDUS can be found on the CTEP Web site (http://ctep.cancer.gov/reporting/cdus.html).

12.2 CTEP Multicenter Guidelines

N/A

12.3 Collaborative Agreements Language

The agent(s) supplied by CTEP, DCTD, NCI used in this protocol is/are provided to the NCI under a Collaborative Agreement (CRADA, CTA, CSA) between the Pharmaceutical Company(ies) (hereinafter referred to as "Collaborator(s)") and the NCI Division of Cancer Treatment and Diagnosis. Therefore, the following obligations/guidelines, in addition to the provisions in the "Intellectual Property Option to Collaborator" (http://ctep.cancer.gov/industryCollaborations2/intellectual_property.htm) contained within the terms of award, apply to the use of the Agent(s) in this study:

1. Agent(s) may not be used for any purpose outside the scope of this protocol, nor can Agent(s) be transferred or licensed to any party not participating in the clinical study. Collaborator(s) data for Agent(s) are confidential and proprietary to Collaborator(s) and shall be maintained as such by the investigators. The protocol documents for studies utilizing Agents contain confidential information and should not be shared or distributed without the permission of the NCI. If a copy of this protocol is requested by a patient or patient's family member participating on the study, the individual should sign a confidentiality agreement. A suitable model agreement can be downloaded from: http://ctep.cancer.gov.

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

    a. NCI will provide all Collaborators with prior written notice regarding the existence and nature of any agreements governing their collaboration with NCI, the design of the proposed combination protocol, and the existence of any obligations that would tend to

restrict NCI's participation in the proposed combination protocol.

b. Each Collaborator shall agree to permit use of the Multi-Party Data from the clinical trial by any other Collaborator solely to the extent necessary to allow said other Collaborator to develop, obtain regulatory approval or commercialize its own Agent.

c. Any Collaborator having the right to use the Multi-Party Data from these trials must agree in writing prior to the commencement of the trials that it will use the Multi-Party Data solely for development, regulatory approval, and commercialization of its own Agent.

3. Clinical Trial Data and Results and Raw Data developed under a Collaborative Agreement will be made available to Collaborator(s), the NCI, and the FDA, as appropriate and unless additional disclosure is required by law or court order as described in the IP Option to Collaborator (http://ctep.cancer.gov/industryCollaborations2/intellectual_property.htm). Additionally, all Clinical Data and Results and Raw Data will be collected, used and disclosed consistent with all applicable federal statutes and regulations for the protection of human subjects, including, if applicable, the *Standards for Privacy of Individually Identifiable Health Information* set forth in 45 C.F.R. Part 164.

4. When a Collaborator wishes to initiate a data request, the request should first be sent to the NCI, who will then notify the appropriate investigators (Group Chair for Cooperative Group studies, or PI for other studies) of Collaborator's wish to contact them.

5. Any data provided to Collaborator(s) for Phase 3 studies must be in accordance with the guidelines and policies of the responsible Data Monitoring Committee (DMC), if there is a DMC for this clinical trial.

6. Any manuscripts reporting the results of this clinical trial must be provided to CTEP by the Group office for Cooperative Group studies or by the principal investigator for non-Cooperative Group studies for immediate delivery to Collaborator(s) for advisory review and comment prior to submission for publication. Collaborator(s) will have 30 days from the date of receipt for review. Collaborator shall have the right to request that publication be delayed for up to an additional 30 days in order to ensure that Collaborator's confidential and proprietary data, in addition to Collaborator(s)'s intellectual property rights, are protected. Copies of abstracts must be provided to CTEP for forwarding to Collaborator(s) for courtesy review as soon as possible and preferably at least three (3) days prior to submission, but in any case, prior to presentation at the meeting or publication in the proceedings. Press releases and other media presentations must also be forwarded to CTEP prior to release. Copies of any manuscript, abstract and/or press release/ media presentation should be sent to:

Email: ncicteppubs@mail.nih.gov

The Regulatory Affairs Branch will then distribute them to Collaborator(s). No publication, manuscript or other form of public disclosure shall contain any of Collaborator's confidential/ proprietary information.

13. STATISTICAL CONSIDERATIONS

13.1 Phase I Study Design/Endpoints

13.1.1 Dose Finding

This phase of the trial is being implemented in order to determine maximum tolerated dose or recommended Phase II of ABT-888 that can safely be delivered with RT in the context of carrying it forward to the efficacy (Phase II) component of this study. The traditional 3+3 dose finding algorithm will be used. Initially 3 patients will be enrolled on dose level 1 and will be observed for dose limiting toxicities during the first 10 weeks (6-7 weeks of radiation therapy followed by a rest period), which is the DLT observation period. If none of these three patients experience a DLT, escalation to dose level 2 will occur. If, on the other hand, 1 out of three patients experience a DLT, then 3 additional patients will be treated at dose level 1. If at most 1 DLT is observed in these 6 patients, escalation to dose level 2 will occur. Otherwise, if 2 or more DLTs are observed in dose level 1, then de-escalation to dose level 0 will take place and the same process will be repeated. The highest dose level with fewer than 2 DLTs in 6 patients will be carried forward to the Phase II component of this study. If dose level 0 is determined to be too toxic (i.e. more than 1 DLT), then the trial will be closed to accrual and the approach will be re-evaluated.

13.1.2 Definition of Evaluable Patient

Patients who receive at least 1 dose of the study regimen and are removed from treatment for toxicity during the dose-finding period are evaluable for estimating the MTD/recommended Phase II dose as long as no additional anti-cancer therapy or supportive care that would confound the interpretation of any observed toxicity or side effect is given.

Patients who receive at least 85% of prescribed therapy during the dose-finding period but who progress prior to completing the rest/evaluation period may be considered evaluable for estimating the MTD, as long as no additional anti-cancer therapy or supportive care that would confound the interpretation of any observed toxicity or side effect is given. Patients should have completed all of the clinical and laboratory monitoring requirements specified by the protocol up to the time of disease progression for them to be considered evaluable for MTD/recommended Phase II dose.

Patients who receive less than 85% of the protocol specified therapy and who go off treatment for reasons other than toxicity (e.g. progressive disease, withdrawal of consent etc.) will be considered inevaluable for estimating the MTD/recommended Phase II and will be replaced.

Patients who complete all therapy during the dose-finding period but who fail to comply with all the specified clinical and laboratory monitoring requirements for the dose finding period may be considered inevaluable by the study chair for estimating the MTD/recommended Phase II and may be replaced.

13.1.3 Feasibility Assessment of Intra-patient Dose Escalation (for patients treated in both Phase I and Phase II of the study):

During maintenance therapy, in an effort to maximize the TMZ dose for each patient, intra-patient dose escalation will be studied. All patients will initiate maintenance treatment with

25 mg/m^2 ABT-888 and 135 mg/m^2 of TMZ with a possibility to escalate to 175 mg/m^2 and to 200 mg/m^2 of TMZ, if no unacceptable toxicities occur following 1 course of treatment at each of these dose levels (**Table 12**). More specifically, intra-patient dose escalation will only be considered if a patient meets the escalation criterion following the first course of TMZ as part of maintenance therapy. Intra-patient dose escalation will be assessed only in patients who are eligible to escalate and escalation to a given dose will be halted based on similar rules employed in traditional 3+3 designs, i.e. if 2 out of first 2-6 patients, 4 out of first 12 patients, etc. experience dose modifying toxicities during their first course of therapy at a given dose level. . This stopping rule will be applied for patients enrolled during the Phase I and Phase II components of the study. Even though these stopping rules will only be enforced during the first course of treatment at a given dose level for TMZ, we will periodically monitor for the occurrence of dose modifying toxicities in later courses and may revise this approach in the event that excessive toxicities are observed.

13.2 Phase II Study Design/Endpoints:

The primary efficacy endpoint of this phase II trial is overall survival defined as the time from initiation of therapy to the date of death from any cause or to the date patient was known to be alive for surviving patients. Progression-free survival (PFS) is a secondary endpoint and is defined as the time from initiation of treatment to the earliest date of failure (disease progression, death from any cause, or second malignancy) for patients who fail and to the last assessment date for patients who have not failed. Patients who start other anti-cancer therapy prior to disease progression will be censored in the Kaplan-Meier estimate of PFS as of the date the alternative therapy began. Patients who have not failed (died) at the time of analyses will be censored at their last date of contact in the Kaplan-Meier estimate of the PFS (overall survival) distribution.

The primary objective of this part of the study will be assessed based on the intent-to-treat (ITT) hypothesis. That is, any eligible patient who receives any ABT-888 will be included in the primary analysis. Note that patients who were treated at the recommended Phase II dose of ABT-888 concurrently with RT during the Phase I component of the study and who meet the eligibility criteria for Phase II will be counted towards the accrual goal of the latter.

13.2.1 Definition of an Evaluable Patient

All patients who receive at least 1 dose of ABT-888 are evaluable for assessing overall survival, the primary endpoint of the Phase II part of this study, as well as for evaluating progression free survival and objective response rates.

13.2.2 Design for the Phase II Component:

As mentioned previously OS is the primary endpoint for the assessment of efficacy of the proposed regimen in patients with newly diagnosed DIPG. The following design utilizes the information available from the PBTC contemporary historical control cohort of eligible patients with newly diagnosed non-metastatic DIPG who were treated on the first five PBTC clinical trials (-006 [phase I, imatinib]; -007 [phase I & phase II, gefitinab]; and -014 [phase I & phase II, tipifarnib]). All patients treated on these trials were between 3 and 18 years of age. Each trial is closed to accrual and all surviving patients have been followed for at least two years to estimate the distribution of OS.

Kaplan-Meier estimate of the distribution of overall survival for the 140 patients treated on the first five trials is shown in the following figure and table.

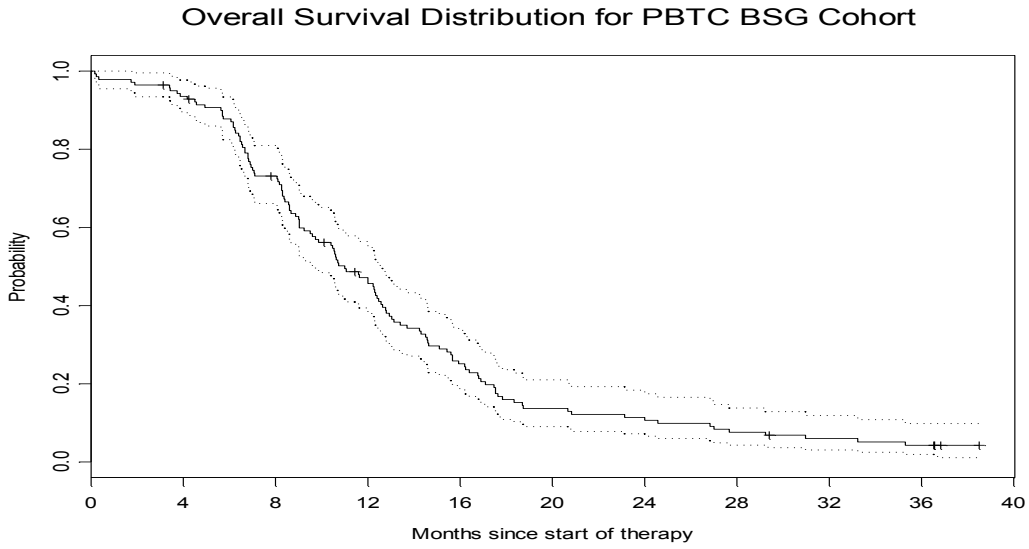
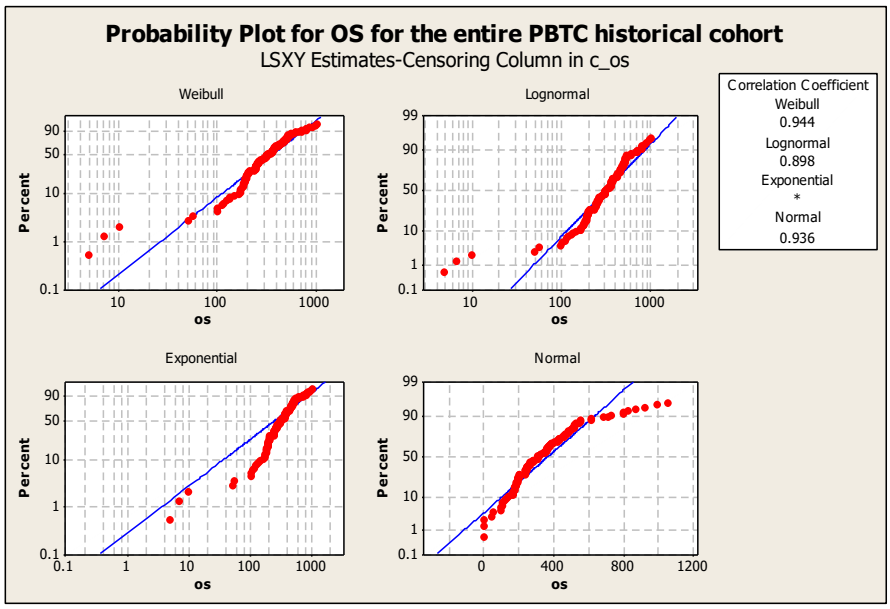


Table 20

Months since Start of Therapy	All Patients				
	# of Risk	Events	Censored	Prob.	SE
0	140	0	0	100.00	0.00
6	140	19	1	86.35	2.90
12	120	59	1	43.75	4.17
18	60	39	0	15.31	3.00
24	21	7	0	10.21	2.50
30	14	5	1	6.56	2.11
36	8	3	0	4.10	1.64
42	5	1	4	2.05	2.03

We will utilize a design approach based on time to event data rather than building the design on a binomial endpoint. Provided a reasonable model could be identified for the outcome data, designs based on time to event endpoints are more informative and make more efficient use of the available data compared to their binomial counterparts both for the purposes of maximizing the overall power of the study and for using the accumulating information for interim monitoring.

The Bayesian approach developed by Thall, Wooten and Tannir[48] will be used for the purpose of determining the sample size as well as establishing a monitoring rule. This approach was developed for monitoring time-to-event outcomes in the context of a single arm Phase II trial. Failure times are modeled via an Exponential distribution with an Inverse Gamma prior on the mean. The approach utilizes periodic monitoring for early stopping. While the OS distribution of the PBTC historical cohort is not exponential, the simulation results presented in the Thall, Wooten and Tannir[48] paper illustrate that the proposed approach is robust in cases where the true distribution is Weibull or Lognormal. As shown in the exploratory plots below, Weibull and the Lognormal distributions seem to fit our historical data best.



The TTEDesigner software available from MD Anderson software download page (https://biostatistics.mdanderson.org/SoftwareDownload/) was used for the necessary calculations.

The prior parameters for the experimental treatment mean were estimated based on the PBTC historical cohort described above. As mentioned previously the Inverse Gamma distribution, which is the conjugate prior for to the Exponential distribution, will be used to provide the prior information on the exponential mean. Besides conjugacy, another attractive aspect of the Inverse Gamma distribution is that its parameters have intuitive interpretations. More specifically in this setting the distribution is parameterized by α_s=number of failures in the historical cohort and β_s=total time at risk in the historical cohort. The values of these parameters were calculated from the PBTC historical control cohort described above. Following the recommended approach by Thall, Wooten and Tannir,[48] for the experimental treatment, we chose a prior that would result in the same median for the experimental treatment as for the historical cohort but with 10 times the variance in the former. This would reflect the *a priori* belief that the two treatments are the same but with much less certainty regarding the experimental treatment. We believe it is reasonable to choose a prior which reflects the belief that the new treatment will lead to a similar outcome as what has been observed to date. If we believe that the experimental treatment is inferior, it would be unethical to conduct this study whereas if we choose a prior that reflects a favorable opinion about the experimental treatment compared to the historical control then such a prior could bias the results in favor of the experimental treatment which is undesirable.
The desired effect size is parameterized in terms of the median time to death. The median time to death estimate based on the current PBTC historical cohort is 10.85 months. Following the Brain Malignancy Steering Committee's recommendation we have set the "effect size" at 5.5 months, reflecting a 50% improvement in the primary outcome parameter, which would certainly be a notable "success", if an experimental treatment were to achieve it.

Our historical experience suggests an average accrual rate of 2 patients per month and we propose to monitor the outcome information twice a year (approximately every 6 months) for early stopping in the event of an unsuccessful treatment. This monitoring schedule coincides

with the PBTC DSMB meetings and hence provides a convenient way of providing up to date outcome information to the PBTC DSMB. With respect to early stopping, the design we present below will only stop for futility. This design has a 6.3% probability of stopping early if the median time to death is 16.5 months for the experimental treatment and 63.85% chance of stopping early if the median time to death is 11 months for the experimental treatment. In the latter case, the mean number of patients expected to be enrolled is 45. So on average we would save 15 patients and 7.5 months of accrual in the event that the experimental treatment is ineffective. Otherwise the trial will enroll 60 patients in approximately 2.5 years. Tables providing the input parameters for design specification and the resulting stopping boundaries associated with the design detailed above are given in **Appendix** G.

Barring early stopping, final analysis will be conducted once all patients have been accrued and followed for at least 2 years or to death, whichever is earlier. This duration of follow-up should be adequate to fully characterize the OS distribution even if the hypothesized 16.5 months for median survival is achieved. As mentioned previously, we will provide KM estimates of the OS distribution, in order to describe outcome information based on the proposed regimen. We will also compare the OS distribution from this trial to the OS distribution of the PBTC historical cohort via the log-rank test to test for a difference. In addition, we will perform a Bayesian analysis of the outcome information providing a 95% credibility interval for the median survival estimate. While it is unlikely, since the prior information is based on the PBTC historical data, if the Bayesian and the frequentist analyses lead to conflicting conclusions, additional analyses will be undertaken to explain the discrepancy but ultimately the frequentist inference will be favored over the Bayesian one.

Under the assumption that the survival distributions are exponentially distributed with the above specified medians and that the analysis time is as detailed above, using the unconditional power estimate proposed by Korn and Freidlin[49] and as implemented in SiZ software package (ver 1.0) the estimated power of this log-rank test based on a 1-sided test and α=0.1 is 87.72%. Since the asymptotic relative efficiency of the log-rank test is 100% in cases where there is no censoring or when there is random but equal censoring in two groups within the family of Weibull distributions[50] we expect that the power estimate above is relatively accurate. As we have indicated previously, the Weibull distribution fits well to our historical control data and given the fact that the final analysis will be done 2 years after the last patient is enrolled we expect that there will be very little censoring in the data. Additionally even though this estimate relies on a normal approximation, Sun, Peng and Tu[51] have shown that when the sample size is larger than 50 the approximation leads to accurate estimates of power. Additionally Sun, Peng and Tu[51] results indicate that the unconditional power formula based on the exponential assumption performs well if the true distribution of the historical control is Weibull. So assuming that the hazards are proportional between our historical cohort and the cohort to be treated on this study, we expect that the above mentioned power estimate to be reasonably accurate.
While the final analysis of the primary objective will be conducted two years after the last patient begins treatment, we will continue to follow all patients for survival information for at least three years or until death, whichever is shorter. Annually this additional information will be available for reporting. At the time of the final analysis for the primary outcome, we will also provide the KM estimate of the PFS distribution. Tumor response refers to the best response prior to failure (disease progression, death from any cause or second malignancy). Exact confidence interval

estimates will be provided for the sustained objective response (PR + CR) rate.
This trial will be monitored by the PBTC DSMB and only the DSMB will have access to summary outcome information until the primary objective of the trial has been achieved. The DSMB meets semi-annually but also meets via teleconferencing if necessary to review and discuss critical events; such as interim analyses. Accrual, demographic and safety data will be reported to the PBTC membership at the PBTC semi-annual meetings.

13.2.3 Statistical Analysis of Pharmacokinetics

Plasma drug concentrations and pharmacokinetic parameters will be presented in tabular and graphical form. Pharmacokinetic parameters of interest, such as apparent volume of the central compartment (Vc/F), elimination rate constant (Ke), half-life (t1/2), apparent oral clearance (CL/F), and area under the plasma concentration time curve (AUC) will be estimated using compartmental methods. Dose proportionality in pharmacokinetic parameters will be investigated by performing one-way analysis of variance (ANOVA) on dose-normalized parameters.

In addition to estimating individual pharmacokinetic parameters, we will also estimate the population parameters using nonlinear mixed effects modeling methods (NONMEM). This method estimates the population parameters and both the inter- and intra-subject variability. Once the population parameters and corresponding covariance matrix are estimated, individual estimates can be obtained using post hoc analysis.

13.3 Projected Accrual Rate and Study Duration

Phase I

The projected accrual rate is 1.5-2.5 patients per month, based on knowledge of the member institutions and their commitment to the Consortium. With 2-3 likely dose levels, the total sample size and the study duration are expected to be 12-18 and about 1.5-2.0 years, respectively. The estimated duration incorporates the planned accrual suspensions for routine toxicity monitoring.

Phase II

The projected accrual rate is 1.5-2.5 patients per month, based on knowledge of the member institutions and their commitment to the Consortium. The total sample size and the study duration are expected to be approximately 60 patients and about 2-3.5 years. The table below is for both phases (Phase I and II combined)

Table 21

Expected: Number of Subjects			
Ethnic Category	**Sex/Gender**		
	Females	**Males**	**Total**
Hispanic or Latino	5	5	10
Not Hispanic or Latino	33	23	56
Ethnic Category: Total of All Subjects *	**38**	**28**	**66**
Racial Categories			
American Indian / Alaska Native	1	0	1

Asian	2	2	4
Native Hawaiian or Other Pacific Islander	0	0	0
Black or African American	5	3	8
White	30	23	53
Racial Categories: Total of All Subjects*	**38**	**28**	**66**

13.4 Analysis of Secondary Endpoints

13.4.1 Statistical Analysis of Imaging Studies:

We will describe and summarize the observed values of the proposed quantitative imaging parameters, namely rCBV, permeability (K^{trans}, v_p, and v_e values) and ADC as well as changes in these parameters via descriptive statistics and plots. If serial values are available from adequate number of patients to make such models meaningful, we will also entertain the use of mixed effects models in modeling changes in these parameters. Depending on the number of pseudoprogression calls observed, we may also explore associations between these imaging parameters and pseudoprogression vs. true progression via appropriate models or simply via summary statistics and descriptive plots within and across patients. The assessment of true vs. pseudo progression will be based on the tumor measurements from subsequent scans. For the primary objective regarding pseudoprogression, we will provide 95% confidence interval estimates of the proportion of patients determined to have experienced pseudoprogression.

13.4.2 Statistical Analysis of Biologic Correlates

13.4.2.1 *NHEJ and PARP Activity*

PARP inhibition in PBMCs will be measured as a ratio of pre/post-treatment values and will be summarized by dose level via exact confidence intervals. The values observed in this trial will be contrasted to the values reported in adults.

Provided adequate number of events is observed among patients who participate in biology studies to make such analyses feasible, we will utilize Cox models to explore associations between the molecular parameters from correlative biology aims 1-2, namely PARP and NHEJ activity or γ-H2AX levels and PFS and OS. Associations between these parameters and objective response may also be explored provided a large enough number of responses are observed. Otherwise correlations between PARP and NHEJ activity or γ-H2AX levels with radiographic response and clinical outcome will be summarized descriptively.

13.4.3 Urine Biomarkers

Comparison of Urine Biomarker Levels in Patients vs. Controls:

In order to determine whether the levels of these urinary biomarkers are elevated in DIPG patients, we will compare median levels for tumor patients to levels in healthy age- and gender-matched controls using the Mann-Whitney U-test. Despite the presence of matched controls, Mann-Whitney U-test allows us to utilize a conservative approach compared to the Wilcoxon signed-rank test since matching by age and gender is done to reduce variability rather than to facilitate the use of methods for paired data. In addition, the percentage of patients and controls above the previously established cut-off values for each urinary biomarker will be compared using the chi-square test. Based on a preliminary power analysis for this chi-square test a sample

size of 58 brain tumor patients and 58 controls will provide 80% power with a conservative two-sided alpha level of 0.01 in capturing a difference of 30% between the groups with respect to each urinary biomarker. We will also utilize ROC analysis for each biomarker in exploring various cut-points for differentiating between the control subjects and DIPG patients. Multivariable logistic regression models coupled with ROC analyses will be used to select a combination of these biomarkers in an attempt to improve their collective performance as a classifier.

Longitudinal Assessment of Changes in Biomarker Values and Their Association with Outcome: Using the data for patients who progress, for each biomarker we will compare the levels at baseline with levels at progression as determined by MRI to assess whether the biomarker values change and if so by how much. This will be done via Wilcoxon signed rank test and exact confidence interval estimates of the percent change. We will also tabulate the percentage of patients who have biomarker levels above the previously established cut points for each biomarker at baseline and at the time of progression. We will investigate whether the percentage of patients who are above/below the cutpoints for each of the biomarkers is different between baseline and time of progression via the McNemar's test. Provided adequate number of responses is present to make such analyses meaningful, we will also perform similar analyses comparing biomarker levels at baseline and at the time of response.

Time-to-event data will be analyzed using the Kaplan-Meier product-limit method and by the log rank test to compare the PFS and OS distributions of patients with biomarker values above and below the specified cut-off values at baseline.[52] Provided adequate number of events exist to make such an exercise informative, will also utilize multivariable Cox proportional-hazards regression model with each of the 8 urinary biomarkers as time dependent predictors and relevant covariates including age and gender to explore associations with PFS and OS.[53]

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Appendix A

Performance Status Criteria

MODIFIED LANSKY SCORE (Score as 0 - 100)

A. Normal Range
- 100 = Fully active
- 90 = Minor restrictions in physically strenuous play
- 80 = Restricted in strenuous play, tires more easily, otherwise active

B. Mild to moderate restriction
- 70 = Both greater restrictions of and less time spent in active play
- 60 = Ambulatory up to 50% of time, limited active play with assistance/supervision
- 50 = Considerable assistance required for any active play; full able to engage in quiet play

C. Moderate to severe restriction
- 40 = Able to initiate quiet activities
- 30 = Needs considerable assistance for quiet activity
- 20 = Limited to very passive activity initiated by others e.g. TV)
- 10 = Completely disabled, not even passive play
- 0 = Unresponsive, coma

KARNOFSKY SCALE
- 100 = Normal; no complaints
- 90 = Able to carry on normal activities; minor signs or symptoms of disease
- 80 = Normal activity with effort
- 70 = Cares for self. Unable to carry on normal activity or to do active work
- 60 = Requires occasional assistance but able to care for most of his/her needs
- 50 = Requires considerable assistance and frequent medical care
- 40 = Disabled; requires special care and assistance
- 30 = Severely disabled; hospitalization indicated though death not imminent
- 20 = Very sick. Hospitalization necessary. Active support treatment necessary.
- 10 = Moribund
- *0 = Dead*

Appendix B

ABT-888 Oral Solution Handling Guidelines

NSC# 737664 ABT-888 (Veliparib)

ABT-888 Oral Solution Dosing Administration
Dosing is based on the BSA at the beginning of radiation and each course of maintenance. The attending physician or designee calculates the required dose in mg based on the patient's BSA and assigned dose. Once the dose is calculated, the attending physician or designee should convert the mg/dose into milliliters; calculate the total milliliters to be dispensed to the family and demonstrate at least once to family member(s) the correct volume of ABT-888 to be drawn and administered per dose. The dose of ABT-888 should be rounded to the nearest 1mg (0.2 ml for the 5 mg/ml concentration).

Care Provider obtains the following from the clinical site:
1. ABT-888 oral solution bottle from the pharmacist at the clinical site.
The ABT-888 oral solution can be used for up to 30 days when stored at room temperature (15°C – 25°C) , protected from light.
2. Oral syringes (5-20 mL). One syringe will be used per dose.

Procedure for Dosing ABT-888 Oral Solution
1. Invert the suspension in the closed bottle before each use.
2. Open the cap and insert the supplied oral dosing syringe into the opening of the press-in bottle adaptor (PIBA), maintaining the bottle in an upright position.
3. Invert the bottle and withdraw the required volume. Your research doctor will tell you how much solution to draw from the bottle for each dose.
4. Remove the oral dosing syringe and administer the content of the syringe (containing solution from Step 3) to patient.
5. Close the bottle tightly and store upright.
6. Place the used syringes in the biohazard bag provided by the research staff. Return the used syringes as well as the ABT-888 solution bottle to the clinic at each visit.

PLEASE FOLLOW STRICTLY ANY ADDITIONAL INSTRUCTIONS GIVEN BY YOUR HOSPITAL'S PHARMACIST

Appendix C

Instructions for Administration of Temodar
(Patients who are unable to swallow capsules)

Temodar is an oral cancer medicine that your child will be taking for treatment of his/her brain tumor. Your child is unable to swallow capsules so the following instructions must be followed for safe administration of this medicine.

- Temodar must be kept in a dark container
- Temodar should be taken the same time every morning, about 60-120 minutes after the morning dose of ABT-888
- If your child requires a nausea medicine, it should be taken prior to the Temodar
- If the dose of Temodar is vomited (which is unusual) within 10 minutes of administration, please repeat the dose. If vomiting occurred more than 10 minutes after a dose, do not repeat the dose
- If the person dispensing this medicine is pregnant or suspects that she is pregnant, she should not dispense this medicine.

Temodar is an anti-cancer agent, and so special precautions must be taken when handling this medicine. There is potential hazard to anyone who handles this medicine once the protective capsule is opened. Since your child is unable to swallow the capsule, you will be required to open the capsules and mix the contents of the capsule in apple sauce or apple juice. This process must be done according to the following guidelines to ensure safe administration of this medicine.

- Find a place that is as free of air flow as possible and is not an area where food is stored or prepared.
- The work surface should be covered with aluminum foil to reduce exposure to other members of the family.
- Temodar can be mixed in apple sauce or apple juice.
- Place the apple sauce or apple juice in a disposable container.
- Put on disposable gloves, mask and goggles (eye protection).
- Open each capsule and place the powder in a medicine cup.
- Add the whole contents of the medicine cup to either apple sauce or apple juice. The medicine will not dissolve completely if mixing in apple juice, and so have extra apple juice on hand so you can add it to any remaining powder in the bottom of the cup.
    - If you need to prepare additional juice or apple sauce, remove your gloves before touching the main container, and then place on new gloves before adding the additional juice or apple sauce to the medicine. (You do not want to contaminate the main container of apple juice/sauce with any powder that may be on your gloves).
- Anything that comes into contact with the medicine must be disposable, such as the spoon used for mixing or eating the apple sauce.
- Once all of the medicine is taken, throw away the following in the red bag: medicine cup, the container the medicine was mixed in, the cover for the work surface, mask, gloves, and anything else that has been in contact with the medicine.
- Once a course of medicine is completed, bring the red bag with you to the clinic so it can be disposed properly.

PLEASE FOLLOW STRICTLY ANY ADDITIONAL INSTRUCTIONS GIVEN BY YOUR HOSPITAL'S PHARMACIST.

Appendix D

ABT-888 Dose Calculation, Capsules (I) During Radiation

(ONLY for Patients with BSA ≥ $1m^2$)

Dose Level : 35 mg/m^2/dose BID				
Min BSA	**Max BSA**	**Total Daily Dose (mg)**	**A.M. Dose (mg)**	**P.M. Dose (mg)**
1.00	1.07	70	40	30
1.08	1.21	80	40	40
1.22	1.35	90	50	40
1.36	1.50	100	50	50
1.51	1.64	110	60	50
1.65	1.78	120	60	60
1.79	1.92	130	70	60
1.93	2.07	140	70	70
2.08	2.21	150	80	70
2.22	2.35	160	80	80
2.36	2.50	170	90	80

Dose Level : 50 mg/m^2/dose BID				
Min BSA	**Max BSA**	**Total Daily Dose (mg)**	**A.M. Dose (mg)**	**P.M. Dose (mg)**
1.00	1.05	100	50	50
1.06	1.15	110	60	50
1.16	1.25	120	60	60
1.26	1.34	130	70	60
1.35	1.45	140	70	70
1.46	1.54	150	80	70
1.55	1.65	160	80	80
1.66	1.74	170	90	80
1.75	1.85	180	90	90
1.86	1.94	190	100	90
1.95	2.05	200	100	100
2.06	2.14	210	110	100
2.15	2.25	220	110	110
2.26	2.34	230	120	110
2.35	2.44	240	120	120
2.45	2.50	250	130	120

Dose Level : 65 mg/m^2/dose BID				
Min BSA	**Max BSA**	**Total Daily Dose (mg)**	**A.M. Dose (mg)**	**P.M. Dose (mg)**
1.00	1.03	130	70	60
1.04	1.11	140	70	70
1.12	1.19	150	80	70
1.20	1.26	160	80	80
1.27	1.34	170	90	80
1.35	1.42	180	90	90
1.43	1.49	190	100	90
1.50	1.57	200	100	100
1.58	1.65	210	110	100
1.66	1.73	220	110	110
1.74	1.80	230	120	110
1.81	1.88	240	120	120
1.89	1.96	250	130	120
1.97	2.03	260	130	130
2.04	2.11	270	140	130
2.12	2.19	280	140	140
2.20	2.26	290	150	140
2.27	2.34	300	150	150
2.35	2.42	310	160	150
2.43	2.50	320	160	160

Dose Level : 85 mg/m^2/dose BID				
Min BSA	**Max BSA**	**Total Daily Dose (mg)**	**A.M. Dose (mg)**	**P.M. Dose (mg)**
1.00	1.02	170	90	80
1.03	1.08	180	90	90
1.09	1.14	190	100	90
1.15	1.20	200	100	100
1.21	1.26	210	110	100
1.27	1.32	220	110	110
1.33	1.38	230	120	110
1.39	1.44	240	120	120
1.45	1.49	250	130	120
1.50	1.55	260	130	130
1.56	1.61	270	140	130
1.62	1.67	280	140	140
1.68	1.73	290	150	140
1.74	1.79	300	150	150
1.80	1.85	310	160	150
1.86	1.91	320	160	160
1.92	1.97	330	170	160
1.98	2.02	340	170	170
2.03	2.08	350	180	170
2.09	2.14	360	180	180
2.15	2.20	370	190	180
2.21	2.26	380	190	190
2.27	2.32	390	200	190
2.33	2.38	400	200	200
2.39	2.44	410	210	200
2.45	2.50	420	210	210

Dose Level : 110 mg/m^2/dose BID				
Min BSA	**Max BSA**	**Total Daily Dose (mg)**	**A.M. Dose (mg)**	**P.M. Dose (mg)**
1.00	1.02	220	110	110
1.03	1.06	230	120	110
1.07	1.11	240	120	120
1.12	1.15	250	130	120
1.16	1.20	260	130	130
1.21	1.24	270	140	130
1.25	1.29	280	140	140
1.30	1.34	290	150	140
1.35	1.38	300	150	150
1.39	1.43	310	160	150
1.44	1.47	320	160	160
1.48	1.52	330	170	160
1.53	1.56	340	170	170
1.57	1.61	350	180	170
1.62	1.65	360	180	180
1.66	170	370	190	180
1.71	1.75	380	190	190
1.76	1.79	390	200	190
1.80	1.84	400	200	200
1.85	1.88	410	210	200
1.89	1.93	420	210	210
1.94	1.97	430	220	210
1.98	2.02	440	220	220
2.03	2.06	450	230	220
2.07	2.11	460	230	230
2.12	2.15	470	240	230
2.16	2.20	480	240	240
2.21	2.24	490	250	240
2.25	2.29	500	250	250
2.30	2.34	510	260	250
2.35	2.38	520	260	260
2.39	2.43	530	270	260
2.44	2.47	540	270	270
2.48	2.50	550	280	270

Appendix E

ABT-888 Dose Calculation, Capsules (II) During Maintenance
(ONLY for Patients with BSA ≥ $1m^2$)

Dose Level : 20 mg/m^2/dose BID				
Min BSA	**Max BSA**	**Total Daily Dose (mg)**	**A.M. Dose (mg)**	**P.M. Dose (mg)**
1.00	1.12	40	20	20
1.13	1.37	50	30	20
1.38	1.62	60	30	30
1.63	1.87	70	40	30
1.88	2.12	80	40	40
2.13	2.37	90	50	40
2.38	2.5	100	50	50

Dose Level : 25 mg/m^2/dose BID				
Min BSA	**Max BSA**	**Total Daily Dose (mg)**	**A.M. Dose (mg)**	**P.M. Dose (mg)**
1.0	1.09	50	30	20
1.1	1.29	60	30	30
1.3	1.49	70	40	30
1.5	1.69	80	40	40
1.7	1.89	90	50	40
1.9	2.10	100	50	50
2.11	2.30	110	60	50
2.31	2.50	120	60	60

Appendix F

Temozolomide Dosing Tables

135 mg/m^2/day					
Min BSA (m^2)	**Max BSA (m^2)**	**Daily TMZ Dose (mg)**	**Min BSA (m^2)**	**Max BSA (m^2)**	**Daily TMZ Dose (mg)**
0.2	0.2	25	1.36	1.38	185
0.21	0.24	30	1.39	1.42	190
0.25	0.27	35	1.43	1.46	195
0.28	0.31	40	1.47	1.49	200
0.32	0.35	45	1.5	1.53	205
0.36	0.38	50	1.54	1.57	210
0.39	0.42	55	1.58	1.61	215
0.43	0.46	60	1.62	1.64	220
0.47	0.49	65	1.65	1.68	225
0.5	0.53	70	1.69	1.72	230
0.54	0.57	75	1.73	1.75	235
0.58	0.61	80	1.76	1.79	240
0.62	0.64	85	1.8	1.83	245
0.65	0.68	90	1.84	1.87	250
0.69	0.72	95	1.88	1.9	255
0.73	0.75	100	1.91	1.94	260
0.76	0.79	105	1.95	1.98	265
0.8	0.83	110	1.99	2.01	270
0.84	0.87	115	2.02	2.05	275
0.88	0.9	120	2.06	2.09	280
0.91	0.94	125	2.1	2.12	285
0.95	0.98	130	2.13	2.16	290
0.99	1.01	135	2.17	2.2	295
1.02	1.05	140	2.21	2.24	300
1.06	1.09	145	2.25	2.27	305
1.1	1.12	150	2.28	2.31	310
1.13	1.16	155	2.32	2.35	315
1.17	1.2	160	2.36	2.38	320
1.21	1.24	165	2.39	2.42	325
1.25	1.27	170	2.43	2.46	330
1.28	1.31	175	2.47	2.49	335
1.32	1.35	180	2.50	2.50	340

175 mg/m^2/day					
Min BSA (m^2)	**Max BSA (m^2)**	**Daily TMZ Dose (mg)**	**Min BSA (m^2)**	**Max BSA (m^2)**	**Daily TMZ Dose (mg)**
0.20	0.21	35	1.36	1.38	240
0.22	0.24	40	1.39	1.41	245
0.25	0.27	45	1.42	1.44	250
0.28	0.29	50	1.45	1.47	255
0.30	0.32	55	1.48	1.50	260
0.33	0.35	60	1.51	1.52	265
0.36	0.38	65	1.53	1.55	270
0.39	0.41	70	1.56	1.58	275
0.42	0.44	75	1.59	1.61	280
0.45	0.47	80	1.62	1.64	285
0.48	0.49	85	1.65	1.67	290
0.50	0.52	90	1.68	1.69	295
0.53	0.55	95	1.70	1.72	300
0.56	0.58	100	1.73	1.75	305
0.59	0.61	105	1.76	1.78	310
0.62	0.64	110	1.79	1.81	315
0.65	0.67	115	1.82	1.84	320
0.68	0.70	120	1.85	1.87	325
0.71	0.72	125	1.88	1.90	330
0.73	0.75	130	1.91	1.92	335
0.76	0.78	135	1.93	1.95	340
0.79	0.81	140	1.96	1.98	345
0.82	0.84	145	1.99	2.01	350
0.85	0.87	150	2.02	2.04	355
0.88	0.89	155	2.05	2.07	360
0.90	0.92	160	2.08	2.09	365
0.93	0.95	165	2.10	2.12	370
0.96	0.98	170	2.13	2.15	375
0.99	1.01	175	2.16	2.18	380
1.02	1.04	180	2.19	2.21	385
1.05	1.07	185	2.22	2.24	390
1.08	1.09	190	2.25	2.27	395
1.10	1.12	195	2.28	2.29	400
1.13	1.15	200	2.30	2.32	405
1.16	1.18	205	2.33	2.35	410
1.19	1.21	210	2.36	2.38	415
1.22	1.24	215	2.39	2.41	420
1.25	1.27	220	2.42	2.44	425
1.28	1.29	225	2.45	2.47	430
1.30	1.32	230	2.48	2.49	435
1.33	1.35	235	2.50	2.50	440

200 mg/m^2/day					
Min BSA (m^2)	**Max BSA (m^2)**	**Daily TMZ Dose (mg)**	**Min BSA (m^2)**	**Max BSA (m^2)**	**Daily TMZ Dose (mg)**
0.20	0.21	40	1.37	1.38	275
0.22	0.23	45	1.39	1.41	280
0.24	0.26	50	1.42	1.43	285
0.27	0.28	55	1.44	1.46	290
0.29	0.31	60	1.47	1.48	295
0.32	0.33	65	1.49	1.51	300
0.34	0.36	70	1.52	1.53	305
0.37	0.38	75	1.54	1.56	310
0.39	0.41	80	1.57	1.58	315
0.42	0.43	85	1.59	1.61	320
0.44	0.46	90	1.62	1.63	325
0.47	0.48	95	1.64	1.66	330
0.49	0.51	100	1.67	1.68	335
0.52	0.53	105	1.69	1.71	340
0.54	0.56	110	1.72	1.73	345
0.57	0.58	115	1.74	1.76	350
0.59	0.61	120	1.77	1.78	355
0.62	0.63	125	1.79	1.81	360
0.64	0.66	130	1.82	1.83	365
0.67	0.68	135	1.84	1.86	370
0.69	0.71	140	1.87	1.88	375
0.72	0.73	145	1.89	1.91	380
0.74	0.76	150	1.92	1.93	385
0.77	0.78	155	1.94	1.96	390
0.79	0.81	160	1.97	1.98	395
0.82	0.83	165	1.99	2.01	400
0.84	0.86	170	2.02	2.03	405
0.87	0.88	175	2.04	2.06	410
0.89	0.91	180	2.07	2.08	415
0.92	0.93	185	2.09	2.11	420
0.94	0.96	190	2.12	2.13	425
0.97	0.98	195	2.14	2.16	430
0.99	1.01	200	2.17	2.18	435
1.02	1.03	205	2.19	2.21	440
1.04	1.06	210	2.22	2.23	445
1.07	1.08	215	2.24	2.26	450
1.09	1.11	220	2.27	2.28	455
1.12	1.13	225	2.29	2.31	460
1.14	1.16	230	2.32	2.33	465
1.17	1.18	235	2.34	2.36	470
1.19	1.21	240	2.37	2.38	475
1.22	1.23	245	2.39	2.41	480
1.24	1.26	250	2.42	2.43	485
1.27	1.28	255	2.44	2.46	490
1.29	1.31	260	2.47	2.48	495
1.32	1.33	265	2.49	2.50	500
1.34	1.36	270			

Appendix G

Design Input Parameters and Resulting Stopping Boundaries

Input Parameters

Parameter	Value
Alpha S	128
Beta S	1759.8
Alpha E	3
Beta E	27.7134
Delta	5.5
pL	0.00807171
Type of Event	Bad
Max patients	60

Stopping Boundaries

Number of events	Minimum total time-on-test (in days) to continue	Number of events	Minimum total time-on-test (in days) to continue	Number of events	Minimum total time-on-test (in days) to continue
1	0	21	10279	41	23262
2	0	22	10904	42	23931
3	216	23	11531	43	24601
4	661	24	12163	44	25273
5	1131	25	12797	45	25946
6	1623	26	13434	46	26619
7	2133	27	14075	47	27295
8	2658	28	14717	48	27971
9	3196	29	15363	49	28648
10	3745	30	16010	50	29326
11	4305	31	16660	51	30005
12	4873	32	17312	52	30686
13	5450	33	17966	53	31367
14	6034	34	18622	54	32049
15	6624	35	19280	55	32732
16	7221	36	19940	56	33416
17	7823	37	20601	57	34100
18	8430	38	21264	58	34786
19	9042	39	21929	59	35472
20	9659	40	22595		