NCI Protocol #: PBTC-047

Local Protocol #: PBTC-047

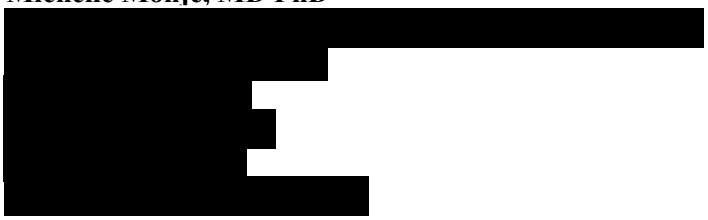
ClinicalTrials.gov Identifier: NCT02717455

TITLE: Phase 1 Trial of Panobinostat in Children with Diffuse Intrinsic Pontine Glioma

Coordinating Center:

Operations, Biostatistics and Data Management Core (OBDMC) for the Pediatric Brain Tumor Consortium (PBTC), St. Jude Children's Research Hospital

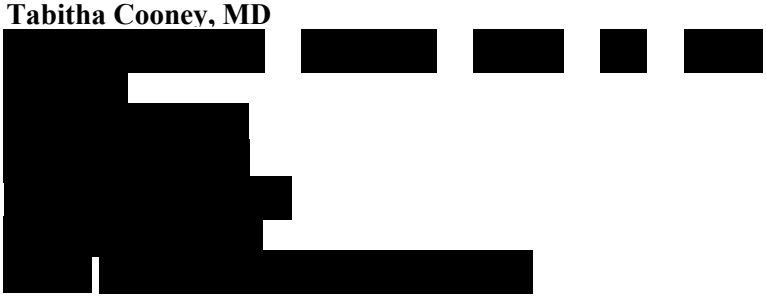
***Principal Investigator:** **Michelle Monje, MD PhD**



Co-Principal Investigators: **Kathy Warren, MD**

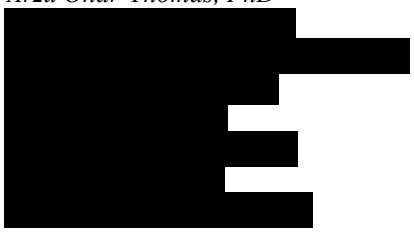
John Glod, M.D.

Co-Investigators: **Tabitha Cooney, MD**

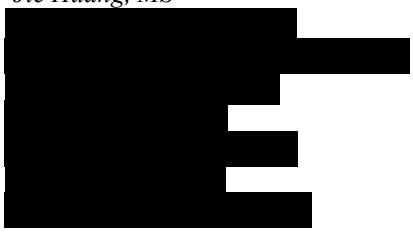


Nada Jabado MD PhD

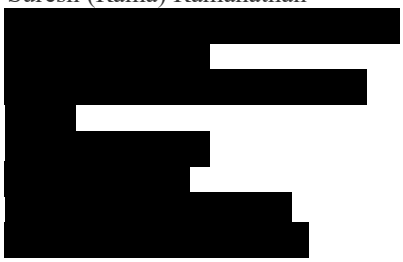
PBTC Biostatistician
Arzu Onar-Thomas, PhD



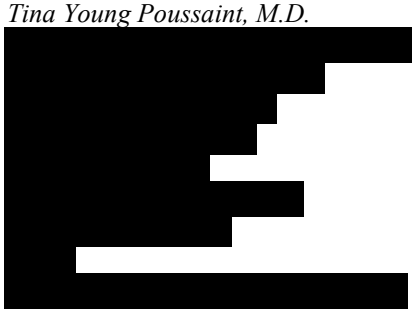
PBTC Biostatistician
Jie Huang, MS



PBTC Protocol Coordinator
Suresh (Rama) Ramanathan



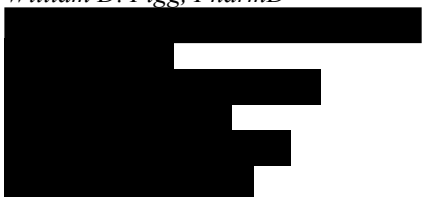
PBTC Neuroimaging Center
Tina Young Poussaint, M.D.



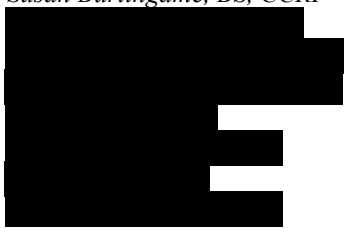
Pharmacodynamics Correlates Laboratory
Michelle Monje MD PhD

Pharmacodynamics Correlates Laboratory
Nada Jabado MD PhD

Pharmacokinetics Laboratory
William D. Figg, PharmD



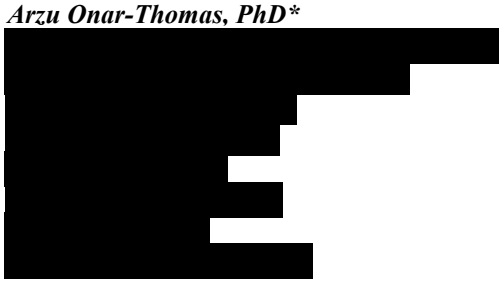
CRA Committee Rep
Susan Burlingame, BS, CCRP



NCI Supplied Agent(s): N/A
Other Agent(s): *Panobinostat (LBH589-lactate)/ Farydak®; NSC #737141; supplied by Secura Bio Inc.*
IND Sponsor: *Center for Cancer Research, NCI/ IND#: 128437*
Protocol Type/ Version#/ Version Date: *Amendment/ Version 8.0/March 26,2021*

Pediatric Brain Tumor Consortium Institutions and Principal Investigators

Arzu Onar-Thomas, PhD*



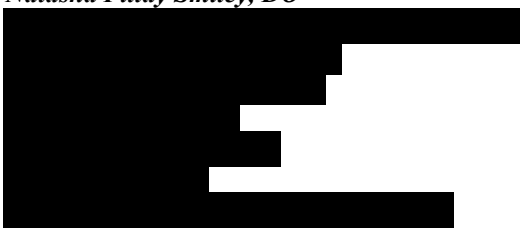
Ira Dunkel, MD

Jason Fangusaro, MD

Eugene Hwang, MD

Alicia Lenzen. MD

Natasha Pillay Smiley, DO



Kathleen Dorris, MD

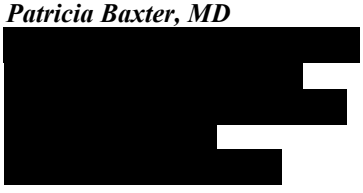
John Glod, MD

Nathan Robison, MD

Giles Robinson, MD

Ian Pollack, MD

Patricia Baxter, MD



Sonia Partap, MD, MS
[redacted]

**Does not have patient care responsibilities*

SCHEMA

Description

This is a multicenter, phase 1 trial of Panobinostat (LBH589) for children with diffuse intrinsic pontine glioma tumors.

Panobinostat is a pan-HDAC inhibitor of Class I, II and IV histone deacetylases (HDACs) involved in the deacetylation of histone and non-histone cellular proteins. Panobinostat inhibits purified total cellular histone deacetylase activity (IC_{50} = 0.03 uM) and activities of most HDAC isoforms (IC_{50} <10nM). In addition, panobinostat induces expression of the cell-cycle control genes including CDKN1A (p21), and selectively inhibits the proliferation of a variety of tumor cells compared to normal cells. It has been extensively profiled for its *in vitro* and *in vivo* pharmacological activity on a variety of tumor cell lines and tumor xenograft mice models.

Based on the *in vitro* and *in vivo* activity of panobinostat in preclinical models using DIPG cell cultures and orthotopic xenograft model systems, and the potentially important role of histone deacetylases and histone 3 K27M mutations in relation to pontine malignancies, we are conducting a Phase 1 study of panobinostat in children with recurrent/progressive DIPG followed by children with DIPG or H3K27+Thalamic Diffuse Malignant Glioma (DMG) prior to progression.

The primary objectives of the study are to (1) describe the toxicity profile and define the dose-limiting toxicities of panobinostat in children with recurrent/progressive DIPG; (2) estimate the maximum tolerated dose and/or the recommended Phase 2 dose of panobinostat in children with recurrent/progressive DIPG; and (3) evaluate and characterize the plasma pharmacokinetics of panobinostat in children with recurrent/progressive DIPG.

Following completion of the first primary objectives, we will pursue investigation of panobinostat with an alternative schedule, in children with DIPG or H3K27+ Thalamic DMG who have not yet progressed. The primary objectives of the amendment are to (1) describe the toxicity profile and define the dose-limiting toxicities of panobinostat in children with non-progressed DIPG or H3K27+ Thalamic DMG treated with three times/week, every other week schedule; (2) estimate the maximum tolerated dose and/or the recommended Phase 2 dose of panobinostat in children with non-progressed DIPG or H3K27+ Thalamic DMG treated with three times/week, every other week schedule; and (3) evaluate and characterize the plasma pharmacokinetics of panobinostat in children with non-progressed DIPG or H3K27+ Thalamic DMG treated with three times/week, every other week schedule.

Schema

Only patients with recurrent or progressive DIPG will be enrolled initially. Panobinostat will be administered every other day, 3 times/week, p.o. preferably on a Monday/Wednesday/Friday schedule for three weeks, followed by a rest period. Three weeks of therapy plus the one week rest period (total 4 weeks) will constitute one course. Treatment will continue for up to two years (26 courses) unless the patient experiences progressive disease, unacceptable toxicity or any of the off-study criteria.

The starting dose (dose level 1) is 10 mg/m^2/day. The table below lists the proposed dose levels to be studied.

Table 1: Dose escalation schedule for recurrent/ progressive DIPG treated with three weeks on, one week off schedule (Stratum 1)

Dose level #	***panobinostat oral dose (mg)***	***Minimum BSA Restriction***
*0**	5 mg/m^2/day MWF, three weeks on, one week off (1 course = 28 days)	Patients must have a BSA ≥ 0.80 m^2
1 (starting dose level)	10 mg/m^2/day MWF, three weeks on, one week off, (1 course = 28 days)	Patients must have a BSA ≥ 0.65 m^2
2	16 mg/m^2/day MWF, three weeks on, one week off, (1 course = 28 days)	Patients must have a BSA ≥ 0.65 m^2
3	22 mg/m^2/day MWF, three weeks on, one week off, (1 course = 28 days)	Patients must have a BSA ≥ 0.65 m^2
4	28 mg/m^2/day MWF, three weeks on, one week off, (1 course = 28 days)	Patients must have a BSA ≥ 0.50 m^2
5	36 mg/m^2/day MWF, three weeks on, one week off, (1 course = 28 days)	Patients must have a BSA ≥ 0.50 m^2
	Panobinostat will be administered as a single agent *** Dose level 0 represents a potential treatment dose for patients requiring a dose reduction from dose level 1 and may be used as a contingency dose level if the starting dose level of panobinostat is not tolerated in the initial cohort.**	

Upon completion of the first primary objectives, patients with DIPG or H3K27+ Thalamic DMG who have received adequate radiation therapy but have not yet progressed will be enrolled in Stratum 2. Panobinostat will be administered every other day, 3 times/week, every other week p.o. preferably on a Monday/Wednesday/Friday schedule. Total 4 weeks will constitute one course. Treatment will continue for up to two years (26 courses) unless the patient experiences progressive disease, unacceptable toxicity or any of the off-treatment criteria.

The starting dose (dose level 1) for Stratum 2 will be 1 dose level above the MTD for the '3-weeks on, 1-week off' schedule. The current expectation is that 10mg/m2 will be the MTD for the '3-weeks on, 1-week off' schedule and thus we expect to start exploring the 'every other week' dosing schedule at 16 mg/m2/day. The table below lists the proposed dose levels to be studied:

Table 2: Dose escalation schedule for non-progressed DIPG or H3K27+Thalamic DMG treated every other week (Stratum 2)

Dose level #	***panobinostat oral dose (mg)***	***Minimum BSA Restriction***
*-1**	5 mg/m^2/day MWF, every other week (1 course = 28 days)	Patients must have a BSA ≥ 0.80 m^2
*0**	10 mg/m^2/day MWF, every other week (1 course = 28 days)	Patients must have a BSA ≥ 0.65 m^2
1 (expected starting dose level)	16 mg/m^2/day MWF, every other week (1 course = 28 days)	Patients must have a BSA ≥ 0.65 m^2
2	22 mg/m^2/day MWF, every other week (1 course = 28 days)	Patients must have a BSA ≥ 0.65 m^2
3	28 mg/m^2/day MWF, every other week (1 course = 28 days)	Patients must have a BSA ≥ 0.50 m^2
4	36 mg/m^2/day MWF, every other week (1 course = 28 days)	Patients must have a BSA ≥ 0.50 m^2
	Panobinostat will be administered as a single agent *** Dose levels 0 and -1 represent potential treatment doses for patients requiring a dose reduction from dose level 1 and may be used as a contingency dose level if the starting dose level of panobinostat is not tolerated in the initial cohort.**	

TABLE OF CONTENTS

1 Objectives

1.1 *Primary Objectives: Recurrent/ Progressive DIPG (Stratum 1)*

1.1.1 To describe the toxicity profile and define the dose-limiting toxicities of panobinostat in children with recurrent/progressive DIPG

1.1.2 To estimate the maximum-tolerated dose and/or the recommended-phase 2 dose of panobinostat in children with recurrent/progressive DIPG

1.1.3 To evaluate and characterize the plasma pharmacokinetics of panobinostat in children with recurrent/progressive DIPG

1.2 *Secondary Objectives: Recurrent/ Progressive DIPG (Stratum 1)*

1.2.1 To describe the progression-free survival (PFS) and overall survival (OS) of children with recurrent or progressive DIPG who are treated with panobinostat

1.2.2 To identify histone 3 K27M mutations in peripheral blood and urine, and evaluate changes with treatment

1.3 *Primary Objectives: Non-progressed DIPG or H3K27M+ Thalamic Diffuse Midline Glioma (DMG) (Stratum 2)*

1.3.1 To describe the toxicity profile and define the dose-limiting toxicities of panobinostat in children with non-progressed DIPG or H3K27M+ Thalamic DMG treated with 3 times/week, every other week.

1.3.2 To estimate the maximum-tolerated dose and/or the recommended-phase 2 dose of panobinostat administered 3 times/week, every other week in children with non-progressed DIPG or H3K27M+ Thalamic DMG

1.3.3 To evaluate and characterize the plasma pharmacokinetics of panobinostat administered 3 times/week, every other week in children with non-progressed DIPG or H3K27M+ Thalamic DMG

1.4 *Secondary Objectives: Non-progressed DIPG or H3K27M+ Thalamic DMG (Stratum 2)*

1.4.1 To describe the progression-free survival (PFS) and overall survival (OS) of children with non-progressed DIPG **or H3K27M+ Thalamic DMG** who are treated with panobinostat

1.4.2 To identify histone 3 K27M mutations in peripheral blood and urine, and evaluate changes with treatment

2 Background

2.1 *Study Disease: Diffuse Intrinsic Pontine Glioma*

Diffuse Intrinsic Pontine Glioma (DIPG) is a devastating, aggressive brain tumor of childhood arising in the ventral pons. Though brainstem tumors are rare among adults, they comprise approximately 10-15% of pediatric brain tumors, with half of all pediatric malignant gliomas occurring in the brainstem[1]. DIPG is the most common tumor subtype in this anatomical region, constituting 80% of brainstem gliomas[2]. With an estimated 200-400 children affected by DIPG annually in the United States, it is the second most common malignant brain tumor of childhood [3]. The prognosis is bleak: in the absence of effective therapies, DIPG is uniformly fatal and is the leading cause of childhood brain tumor death. Median age at diagnosis is 6-7 years, with median survival of 9 months; 90% of children will die from the disease within 2 years of initial diagnosis, with less than 1% surviving after 5 years[4].

Because DIPG grows diffusely and infiltrates critical brainstem structures, surgical resection is not possible. Radiation therapy has remained the mainstay of treatment for the past three decades since its introduction. At most treatment centers, the standard recommendation is conventionally fractionated local field radiotherapy with dose range of 54-60 Gy for a period of 6 weeks[5]. Radiotherapy provides temporary improvement or stabilization of symptoms and extends overall survival by an average of 3 months; median survival is less than 5 months without radiation[6]. Though both clinical and radiographic responses are initially observed, local recurrence invariably occurs.

Many clinical trials of the past three decades have explored the use of various chemotherapeutic agents for DIPG, employing conventional and high-dose strategies as well as targeted agents. Chemotherapy has been attempted at time points before, during and after radiation therapy. Despite all efforts, no improvement in overall survival has been demonstrated[7],[8],[9],[10],[11],[12],[13],[14]. No chemotherapeutic agent has ever shown significant efficacy for DIPG. However, these trials were performed largely without guidance by direct preclinical experimental data for DIPG; rather, they were designed as analogues of therapeutic strategies used for adult high-grade glioma. This approach is problematic: although adult and pediatric high-grade gliomas may lie on the same disease spectrum with certain shared histopathologic features, emerging research suggests that DIPG exemplifies a distinct biology from adult malignant glioma. Preclinical data for adult high-grade glioma thus cannot be assumed to generalize to DIPG.

In recent years, a rapid expansion in our understanding of the biology of DIPG has occurred alongside the expansion of available autopsy/biopsy tissue, development of DIPG cell lines, and animal models for molecular analysis. The availability of this material, paired with the advent of next generation sequencing tools, has enabled groundbreaking new research revealing a complex genomic and epigenetic landscape that characterizes DIPG as a unique entity, distinct from high-grade gliomas that arise in adulthood, and even distinct from pediatric supratentorial malignant gliomas.

Perhaps most revolutionary in our understanding of the molecular mechanisms of DIPG pathogenesis has been the discovery that most (78%) DIPG tumors contain a specific, recurrent mutation in one of two genes encoding histones, key chromatin components that play important

roles in regulating the epigenome[15,16]. A high fraction of diffuse midline gliomas of the thalamus carry the same H3K27M+ mutation, and the World Health Organization (WHO) now classifies diffuse midline gliomas of the pons and thalamus with the H3K27M mutation as the same disease. About 60-75% of these identified histone mutations occur in *H3F3A* (H3 histone, family 3A), a gene encoding histone variant H3.3, which replaces histones as necessary in the event of nucleosome disruption[17,18]. Another identified recurrent mutation, mutually exclusive of mutant *H3F3A*, is known to affect histone variant H3.1, usually via an alteration in *HIST1H3B* (histone cluster 1, H3b) and very rarely in *HIST1H3C* (histone cluster 1, H3c)[19,20]. Histone H3.1 plays a role in packaging newly synthesized DNA during S-phase[18]. In both H3.3 and H3.1, the alteration is a specific missense mutation resulting in the substitution of lysine with methionine at position 27 (K27M)[15,16]. This position is located within the N-terminal tail of the histone; importantly, post-translational modification of histone tails by methylation, acetylation or ubiquitylation of lysine residues is known to mediate the epigenetic regulation of gene expression and alter nucleosome structure. The addition or deletion of such modifications are facilitated by "writers" and "erasers" and result in altered interactions with transcription modifiers, as mediated by "readers." While mutations in writers, erasers or readers have recently been implicated in other oncogenic pathways, it appears that in DIPG the epigenetic aberrancy directly results from mutation of the histone alone[21,22,23,24]. Indeed, DIPG represents the first identified example of the implication of a histone mutation in oncogenesis and disease[22,25,26,27]. Remarkably, the H3K27M mutation is heterozygous in 100% of DIPG cells, and remains so in both treatment-naïve and treatment-exposed samples, and within low- and high-grade tumor regions[21,22,28,29,23,24]. This strongly suggests clonal selection, emphasizing the robust selective advantage that the H3K27M mutation likely confers.

H3K27M is a gain-of-function mutation that exerts broad transcriptional effects by disrupting lysine trimethylation at position 27. The K27 trimethylation mark (H3K27me3) is necessary for stimulation of methyltransferase activity of Polycomb Repressive Complex 2 (PRC2) via nucleosome interaction with the EZH2 subunit of PRC2[30]. PRC2 is known to silence gene transcription in order to regulate stem cell differentiation in development, and mutations in subunits of PRC2 itself have been previously implicated in oncogenesis[31]. The substitution of lysine with methionine at position 27 interferes with PRC2 stimulation and results in the robust, aberrant derepression of gene transcription normally silenced by PRC2 activity[30]. In DIPG samples, this occurs in the absence of altered EZH2 expression[32,33]. Strikingly, while the mutant H3 variants represent only a fraction (~3-17%) of the total histone H3 population in DIPG cells, this mutation nonetheless exerts a dominant reprogramming effect and initiates a global pattern of hypomethylation and increased transcriptional activity, with dramatic loss of trimethylation or dimethylation observed across all H3 variants in human DIPG samples as well as in *in vivo* and *in vitro* models[34,35,30,32]; ectopic H3K27M expression in other cell types similarly leads to global reduction of H3K27me3[30,33]. Certain trimethylation marks remain, but these were found to associate with certain target genes not usually under H3K27me3 control, possibly signifying further alteration of transcription regulation[33,36]. Interestingly, there is also an observed increase in H3K27me3 in regions simultaneously trimethylated at H3K4, a mark that usually promotes active gene expression[36]. This contradictory combination of "silent" and "active" marks signifies that the associated target genes are "bivalent" - i.e., uniquely primed for expression upon H3K27 trimethylation loss—and indeed, these target genes were found to be involved in oncogenic as well as developmental pathways[36]. This evidence of broad transcription alterations occurring in such a

vast majority of DIPG tumors provides compelling support for an epigenetic mechanism as a key driver of DIPG pathogenesis.

2.2 *Inhibition of Deacetylase*

Panobinostat (LBH589) is a deacetylase inhibitor (DACi) belonging to a structurally novel cinnamic hydroxamic acid class of compounds. It is a potent class I/II pan-DAC inhibitor (pan-DACi) that has shown anti-tumor activity in pre-clinical models and cancer patients. Deacetylases (DAC) target lysine groups on chromatin and transcription factors and various non-histone proteins such as p53, tubulin, HSP90 and Rb.

Inhibition of DAC provides a novel approach for cancer treatment. Histones are part of the core proteins of nucleosomes, and acetylation and deacetylation of these proteins play a role in the regulation of gene expression. Highly charged deacetylated histones bind tightly to the phosphate backbone of DNA, inhibiting transcription, presumably, by limiting access of transcription factors and RNA polymerases to DNA. Acetylation neutralizes the charge of histones and generates a more open DNA conformation. This conformation allows transcription factors and associated transcription apparatus access to the DNA, promoting expression of the corresponding genes. The opposing activities of two groups of enzymes, histone acetyltransferase (HAT) and HDAC control the amount of acetylation. In normal cells a balance exists between HAT and DAC activity that leads to cell specific patterns of gene expression. Perturbation of the balance produces changes in gene expression.

Several lines of evidence suggest that aberrant recruitment of HDAC and the resulting modification of chromatin structure may play a role in changing the gene expression seen in transformed cells. DAC inhibitors (DACi) have been shown to induce differentiation, cell cycle arrest or apoptosis in cultured tumor cells, and to inhibit the growth of tumors in animal models [37,38,39, 40, 41,42,43]. In addition, DACi have been shown to induce expression of p21, a key mediator of cell cycle arrest in G1 phase and cellular differentiation[44, 45, 46, 47].

Tumor growth inhibition and apoptosis in response to DACi treatment may also be mediated through changes in acetylation of non-histone proteins (e.g., HSP90, p53, HIF-1α, α-tubulin). For example, the chaperone protein HSP90 has been shown to be acetylated in cells treated with DACi[48, 49, 50]. Acetylation of HSP90 inhibits its ability to bind newly synthesized client proteins, thus preventing proper client protein folding and function. In the absence of HSP90 function, misfolded proteins are targeted for degradation in the proteasome. Many proteins that require HSP90 association are critical to cancer cell growth, including ErbB1, ErbB2, AKT, Raf, KDR, and BCR-ABL. Acetylation of HSP90 in cells treated with DACi inhibits the chaperone function of HSP90, leading to degradation of the client proteins and eventual cell death.

Given the presence of histone mutations in the vast majority of DIPGs and the anti-tumor effects of HDAC inhibitors, there is strong rationale to evaluate the histone deacetylase inhibitor, Panobinostat, against DIPG.

2.3 *Panobinostat (LBH589)*

Panobinostat is a pan-HDAC inhibitor of Class I, II and IV histone deacetylases (HDACs) involved in the deacetylation of histone and non-histone cellular proteins. Panobinostat inhibits purified total cellular histone deacetylase activity (IC_{50} = 0.03 uM) and activities of most HDAC isoforms (IC_{50} < 10nM). In addition, panobinostat induces expression of the cell-cycle control genes including CDKNIA (p21), and selectively inhibits the proliferation of a variety of tumor cells compared to normal cells. It has been extensively profiled for its *in vitro* and *in vivo* pharmacological activity on a variety of tumor cell lines and tumor xenograft mice models.

In Vitro Studies

Panobinostat was profiled for antiproliferative activity in a large panel of solid and hematological malignancy cell lines and subsets of tumor cell types exhibiting sensitivity or relative insensitivity to the drug were identified. All leukemia and lymphoma cell lines tested were highly sensitive to panobinostat (LD50 < 50 nM). Significant antiproliferative activity was observed with panobinostat in combination with relevant standard of care agents in plasma cells isolated from patients with MM and in leukemia cell lines[51, 52,53]. Exposure of normal fibroblasts to low nanomolar concentrations of panobinostat inhibited their proliferation; however, treatment with up to micromolar concentrations of the compound for 72 hours did not induce significant cell death. Panobinostat-induced apoptosis of bronchial epithelial cells transformed by SV40 large T antigen/telomerase, but had little effect on normal bronchial epithelial cells, even at greater concentrations. These results provide the basis for a potentially favorable therapeutic window during anticancer therapy with panobinostat. Cultured tumor cells and/or tumors treated with panobinostat exhibit increased levels of acetylated histones, indicating that the anti-tumor activity of panobinostat is accompanied by HDAC inhibition in cell lines and in vivo.

In Vivo Studies

Human xenografts of tumor cell lines growing in athymic nude mice were used to profile panobinostat *in vivo* in several models. Panobinostat demonstrated single agent and/or combination activity in a range of xenograft models including colon, SCLC, cutaneous T-cell lymphoma (CTCL), multiple myeloma (MM) and other xenografted human primary tumors[51]. Single agent treatment in HCT116 xenograft tumors (colorectal) resulted in dose-related anti-tumor activity with minimal toxicity at panobinostat concentrations which correlated with persistent histone acetylation. Inhibition of tumor growth and tumor regression with minimal toxicity, as assessed by animal body weight, was also observed in the HH CTCL xenograft model and the NCI-H146 SCLC xenograft model. Panobinostat also demonstrated significant anti-tumor activity in androgen-independent prostate cancer xenograft model, in two different types of human plasmacytoma murine models, in combination with trastuzumab in the HER+ BT474 breast cancer tumor xenograft model (data on file), in combination with docetaxel in the HID28 hormone independent prostate cancer model [Study RD-2010-50257].

Mechanism of Action

Although a known multi-HDAC inhibitor, the exact anti-tumor mechanism of action is not fully

elucidated and may include effects on epigenetic mechanisms of gene expression as well as modulation of acetylation and function of histones. Panobinostat increases histone acetylation in preclinical models as well as in exploratory pharmacodynamic assessments on patient samples in clinical trials. Panobinostat treatment results in the induction of cell-cycle control genes such as p21, which is associated with induction of cell-cycle arrest. Treatment of tumor cells with panobinostat results in increased acetylation of cytoplasmic proteins, including Hsp90, a target of HDAC6 and a major chaperone protein required for the stabilization of many key proteins implicated in cancer development and growth onco-proteins. Panobinostat-induced acetylation of Hsp90 inhibited the protein-stabilizing function of Hsp90 and led to the destabilization and resulting degradation of tumor targets. As described below, in H3K27M mutant cells (such as most DIPGs) panobinostat-induced acetylation of histone-3 results in normalization of histone-3 trimethylation, likely by blocking the sequestration of PRC2 by the mutant methionine in the oncohistone. This normalization of H3K27 trimethylation in the wild type histones results in sweeping normalization of the H3K27M gene expression signature[54].

2.3.1 DIPG Preclinical Efficacy Data

DIPG research has been limited historically by a dearth of tumor tissue available for study and lack of experimental model systems. In recent years, however, this has begun to change. Employing autopsy[55,58,56] and biopsy[57] tissue, patient-derived DIPG cell cultures and orthotopic xenograft model systems have recently been established, with the first available xenograftable DIPG cell culture reported in 2011[58, 56, 57]. Armed with the tools to design clinical trials based on preclinical data, an international, multi-institutional collaboration formed to pool DIPG tissue resources and evaluate functional targets for DIPG therapy. This DIPG Preclinical Consortium performed a drug screen using a total of 16 DIPG cell cultures and found panobinostat to be among the top hits. 12/16 DIPG cell cultures exhibited sensitivity to panobinostat, with an IC_{50} of ~100 nM[54]. Panobinostat was found to decrease DIPG cell proliferation and increase DIPG cell death (**Figure 1**a-b). Further, the increase in histone acetylation caused by panobinostat exposure correlated with an unexpected normalization of histone 3 K27 methylation in H3.3K27M mutant DIPG cells or 293T cells expressing an H3.3K27M construct[54] (**Figure 1**c). Accordingly, a normalization of the "K27M" gene expression signature was observed[54].

Panobinostat was directly infused into the pons in a pontine orthotopic xenograft model of DIPG (SU-DIPG VI; H3.3K27M subtype) via convection-enhanced delivery (CED). *In vivo* bioluminescent imaging was performed immediately prior to and 7 days after panobinostat (or vehicle control) administration in order to measure tumor growth by quantitative assessment of photon emission (**Figure 1**d-g). Because panobinostat affects gene expression, we confirmed that panobinostat expression does not down-regulate the luciferase transgene. We found a marked effect of panobinostat on the rate of tumor xenograft growth; in vehicle-treated control mice, the rate of xenograft growth was approximately 6.5 fold greater than that in mice treated with a single dose of panobinostat by CED (n=4 vehicle controls, 5 treated mice; $P<0.05$ by two-tailed t test; **Figure 1**g). Having shown proof of principle for *in vivo* efficacy of panobinostat against DIPG, we next investigated the degree to which panobinostat penetrates the pons when administered systemically using liquid chromatography-tandem mass spectrometry (LC-MS/MS). Following a single 20 mg/kg IP dose, we found pontine panobinostat levels of 0.068ng/mg tissue, equivalent to ~200 nM. As the IC_{50} for panobinostat was found to be ~100 nM, we reasoned that systemic

delivery may prove effective. Mice with brainstem orthotopic xenografts of SU-DIPG-VI cells were then treated with 1 mg/kg, 10 mg/kg or 20 mg/kg IP panobinostat. *In vivo* bioluminescent imaging demonstrated a significant reduction in tumor growth in panobinostat-treated animals at 1 week in 10 mg/kg and 20 mg/kg treatment groups compared to vehicle-treated controls (n=5 per group and 7 per group; P<0.05 and 0.01 by two-tailed t test, respectively; **Figure 1**h). A second, patient-derived DIPG brainstem orthotopic xenograft model (IBs-w0128DIPG, derived from case LI-F; H3WT subtype) was also tested using panobinostat 10 mg/kg IP dosed 5 days on, 5 days off, for a total of 9 doses. This resulted in significantly prolonged survival in the mice treated with panobinostat compared to vehicle treated controls (**Figure 1**, n=10 per group, $P < 0.05$ by log-rank analysis).

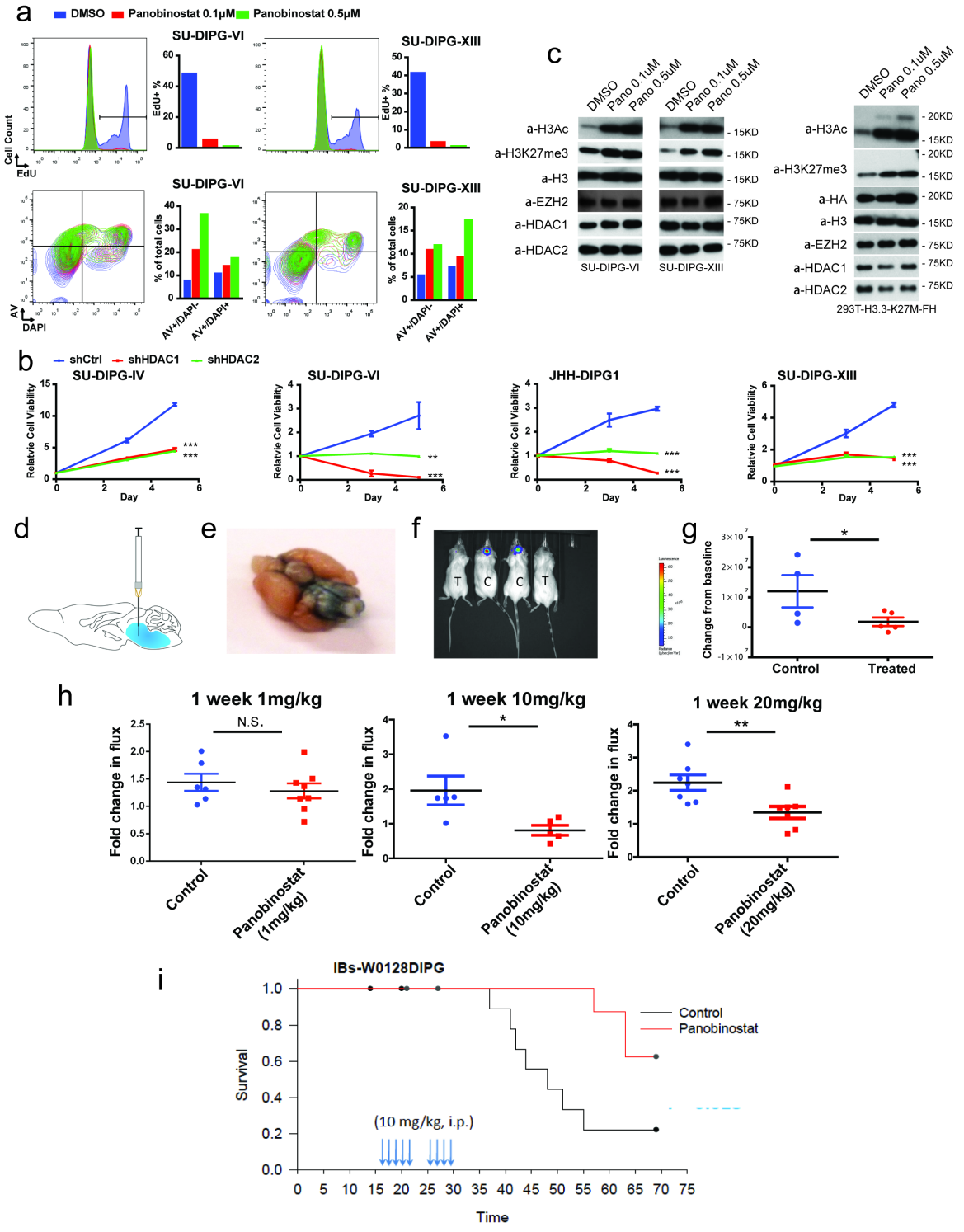


Figure 1

Preclinical Panobinostat activity against DIPG

(**a**) FACS analysis of DIPG tumor cell proliferation and cell death: Top row: Overlapping

histogram plots of EdU FACS analyses are shown on the left; quantifications of EdU^+ cell population levels from each condition are shown in bar plots on the right for DIPG cell cultures SU-DIPG-VI and SU-DIPG-XIII (both H3.3K27M mutant cell lines). Bottom row: Left, Overlapping plots of Annexin V, DAPI FACS analyses; Right, bar plots show early apoptotic (AV^+, $DAPI^-$) or late apoptotic (AV^+, $DAPI^+$) cell population levels from each condition for each cell line as above. (**b**) *HDAC1* and *HDAC2* knock-down in DIPG cells using shRNA verify panobinostat mechanism of action in four DIPG cell lines. Cell viability assays at each time point for each cell line were performed in triplicate (n = 3 wells); data are expressed relative to Day 0 and are shown as mean ± SD. Note the varying growth rates of cell cultures result in varying y-axes depicting relative change in cell viability. **P < 0.01; ***P < 0.001 (Two-way ANOVA). (**c**) Panobinostat increases histone-3 acetylation and restores H3K27 trimethylation. Western blot analyses of histone-3 acetylation and H3K27 trimethylation (H3K27me3) in H3K27M mutant DIPG cell lines SU-DIPG-VI and SU-DIPG-XIII (left blots) and in 293T cells expressing a mutant H3.3K27M-HA tagged construct (293-H3.3-K27M-FH[36]; right blots). Controls included total protein levels of H3, HDAC1, HDAC2 and EZH2. Expression of the HA tag in the 293T cells confirms expression of the H3.3K27M-FH construct. (**d**) Schematic illustrating convection enhanced delivery strategy to infuse drug into brainstem. Blue illustrates approximate distribution of the infused solution. (**e)** Distribution of infusate illustrated by delivering blue dye to the brainstem by CED. Ventral side of a mouse brain is shown immediately following CED delivery of Coomassie Blue dye. Scale bar = 3 mm (**f**) *in vivo* bioluminescent imaging of DIPG xenografts 7 days following CED delivery of panobinostat (T = treated with panobinostat) or vehicle control (C = control). The heat map superimposed over the mouse head represents the degree of photon emission by DIPG cells expressing firefly luciferase. Scale bar = 3.5 cm. (**g)** *in vivo* DIPG xenograft tumor growth as measured by change in bioluminescent photon emission over the seven days following (**g**) CED delivery of panobinostat. Panobinostat = red squares (n = 5 mice) and vehicle control = blue circles (n = 4 mice). Data points represent the change in maximum photon flux (percent of baseline) between Day 0 and Day 7 for each mouse. (**h**) As in (g), with systemic administration of panobinostat. Three systemic dose levels were used, 1 mg/kg (n = 6 control, 8 treated mice) or 10 mg/kg (n = 5 mice per group) delivered IP on M,W,F or 20 mg/kg (n = 7 mice per group) delivered once per week. Error bars, s.e.m. *P < 0. 0.5; **P < 0.01; N.S. indicates P > 0.05 (two-tailed t test). (**i**) Systemic delivery of panobinostat prolongs survival in a histone H3 wild type DIPG orthotopic xenograft model IBs-W0128DIPG. Panobinostat 10 mg/kg I.P. doses given as indicated by arrows. n = 10 per group; P = 0.0179 (log-rank analysis).

Biodistribution of Panobinostat

We carefully tested brain penetration of panobinostat in rodents. Non-tumor bearing mice were given a dose of panobinostat (20 mg/kg IP or 10 mg/kg PO), and levels of panobinostat were measured in the brain parenchyma (following transcardiac perfusion of saline to avoid sample contamination by blood levels of the drug) 30 minutes later using liquid chromatography-tandem mass spectrometry (LC-MSMS). In the pons, we measured drug level of ~.068 ng/mg (equivalent to ~196 nM) following 20 mg/kg IP dosing[54]. Because 20 mg/kg is a relatively high dose and was administered IP, to better model the MTD described in the TACL study we used 10 mg/kg PO dosing and measured at 30 min following this PO dose a concentration of ~0.053 ng/mg in hindbrain (brainstem plus cerebellum) tissue (unpublished results). While these brain concentrations of panobinostat are only a fraction of what is measured in the serum from the same

mice, they are greater than the DIPG cell IC50 for panobinostat of ~100 nM. Accordingly, when we treat mice bearing patient-derived DIPG orthotopic xenografts with panobinostat systemically 3 times per week (10 mg/kg), we still measured a decrease in tumor growth and an increase in survival, albeit the duration of suppressed growth was less than in the mice treated with weekly 20mg/kg systemic panobinostat[54]. In summary, our preclinical data suggest that while very little panobinostat penetrates the brain parenchyma, DIPG cells are sufficiently sensitive to it that enough reaches the brain for tumor cell killing to occur.

2.3.2 Animal Toxicity

A complete toxicity safety evaluation program of acute, subchronic, chronic, and reproductive toxicology studies and genotoxicity studies was conducted to support the chronic administration of panobinostat to adult patients. The toxicology program was consistent with the International Conference on Harmonization (ICH) M3 Guideline on Non-clinical Safety Studies for the Conduct of Human Clinical Trials for Pharmaceuticals as well as all other relevant ICH Guidelines on Safety. All pivotal toxicology studies (**Table 3**) were performed in accordance with GLP guidelines and currently accepted guidelines with respect to animal numbers and dose levels used.

Table 3

Pivotal GLP toxicity studies

Study Type and Duration	Species	Route	Dose Groups mg/kg
Single-dose LD_{50}	Mouse	i.v.	0, 10, 50, 75,100
Single-dose LD_{50}	Rat (Wistar)	i.v.	0, 1, 10, 50, 100
4-week	Rat (Wistar)	Oral	0, 3, 10, 30
13-week	Rat (Wistar)	Oral	0, 10, 30, 100
26-week	Rat (Wistar)	Oral	0, 10, 30, 75
1-week	Rat (Wistar)	i.v.	0.9, 2
4-week	Rat (Wistar)	i.v.	0.05, 0.3, 0.9
13-week	Rat (Wistar)	i.v.	0.3, 1.0, 3.0
4-week	Dog	Oral	0, 0.15. 0.5, 1.5
13-week	Dog	Oral	0,0.15, 0.5, 1.5/1.0
39-week	Dog	Oral	0, 0.15, 0.5, 1.0
4-week	Dog	i.v.	0.06, 0.2, 0.6
13-week	Dog	i.v.	0.2, 0.6, 1.2
Ames assay	Salmonella typhimurium	In vitro	4-5000 µg/plate +/- S9
Chromosome aberration	Human lymphocytes	In vitro	0.1-21.0 µg/ml
Fertility	Rat (Wistar)	Oral	0, 10, 30, 100
Embryo-fetal development	Rat (Wistar)	Oral	3, 10, 30
Embryo-fetal development	Rat (Wistar)	Oral	30, 100, 300
Embryo-fetal development	Rabbit (NZW)	Oral	10, 40, 80
Phototoxicity	3T3 Fibroblasts	In vitro	Photo Irritation Factor =1.3
Contact sensitization LLNA	Mice	In vivo	1 %, 5 % and 10 % w/w
Local tolerance	Rabbit	i.v.	0.234 mg per injection

Single Dose I.V. Toxicity Studies
The maximum non-lethal dose following a single i.v. administration of panobinostat was between

50-75 mg/kg in mice and between 10-50 mg/kg in rats.

Table 4

Summary of single dose toxicity studies

Study Type/ Study no.	Species/ N/sex/group	Doses (mg/kg)/ Dose volume/ Batch no.	Major Findings
Acute (0270147) GLP	Mice(CD-1)/ 3-5/sex/group	0, 10, 50, 75,100 mg/kg 20 mL/kg (20% propylene glycol/80% buffer) Batch no. 0251001	100 mg/kg: mortality (1/3 m and 3/5 f) 75 mg/kg: mortality (2/5 m) ≥ 50 mg/kg: clinical signs (↓ feces, ↓ locomotor activity) ≥ 10 mg/kg: body weight loss non-lethal dose: 50 mg/kg (m), 75 mg/kg (f)
Acute (0270146) GLP	Rats(Wistar)/ 5/sex/group	0, 1, 10, 50, 100 mg/kg 10 mL/kg (20% propylene glycol/80% buffer) Batch no. 0251001	100 mg/kg: 100% mortality ≥ 50 mg/kg: mortality (2/5 f) ≥ 10 mg/kg: body weight loss non-lethal dose: 50 mg/kg (m), 10 mg/kg (f)

Repeat Dose Toxicity Studies

The primary target organs of toxicity following repeat dose i.v. and oral administration to rats and dogs are the hematopoietic and lymphatic systems. Decreases in red cell parameters and white blood cell counts (lymphocyte, neutrophil, eosinophil, monocyte, and basophil counts) were seen following multiple doses. Reductions in red blood cell parameters and hemoglobin were accompanied by marginally increased reticulocyte counts at high doses in rats and dogs. Platelet counts were decreased in rats but increased in dogs. Panobinostat causes a cessation of platelet production and maturation rather than megakaryocyte loss, and platelet generation resumes rapidly upon discontinuation of treatment. Recovery involves a rebound effect with increased megakaryocyte generation and rapid thrombopoiesis[58,59].

Diarrhea and necrosis of the epithelium in the small intestine was observed in dogs and rats following both oral and intravenous administration. In rats, GI changes were present at dose levels that exceeded the maximum tolerated dose. These findings are not unexpected since HDACs play a role in the maintenance of intestinal stem-cell proliferation, survival and differentiation and degenerative changes of the intestinal tract are common findings with all HDAC inhibitors.

The thyroid was identified as a target organ for panobinostat based upon variable occurrences of thyroid weight changes, decreased colloid, follicular epithelial hypertrophy and cytoplasmic vacuolation. Effects on the thyroid were observed in rats and dogs following both oral and i.v. administration of panobinostat. A NOAEL (No-observed-adverse-effect level) was not identified.

Hyperostosis of the femoral cavity was observed in the 13-week oral rat study. However, no adverse bone effects were observed in chronic studies in rats and dogs. These effects are likely to be pharmacologically mediated since HDAC inhibitors have been shown to promote osteoblast maturation *in vitro*, accelerate osteogenesis, and suppress osteoclastogenesis and bone destruction

in rats.

Genotoxicity / Mutagenicity Studies

Panobinostat demonstrated mutagenic potential in bacterial cells and endoreduplication effects in eukaryotic cells in vitro that are attributed to the pharmacological mode of action. According to current classification criteria for mutagenicity, panobinostat is classified as a category 4 substance.

Panobinostat was tested for genotoxic activity in vitro. The AMES test and the chromosomal aberration (COMET) test were conducted according to GLP procedures. Study design and results are summarized in **Table 5**.

Table 5

Summary of genotoxicity studies

Test System	Species/strain/cell line	Concentrations	Major Findings
AMES screen	S. typhimurium: TA98, TA100	15-5000 µg/plate (+/- S9)	Mutagenic in strain TA98 without S9 at ≥ 1250 µg/plate
AMES test	S. typhimurium: TA1535, TA97a, TA98, TA100, TA102	4-5000 µg/plate (+/- S9)	Mutagenic in strains TA1535 and TA97 at ≥ 625 µg/plate
Comet screen	L5178Y mouse lymphoma cells	55.5-222.2 µg/ml (-S9) 20.9-166 µg/ml (+S9)	Induction of DNA damage
Chromosome Aberration	Human peripheral blood lymphocytes	0.6-2.4 µg/ml (-S9, 20h treatment) 0.04-0.44 µg/ml (-S9, 3h treatment + 17h recovery) 0.6-9.4 µg/ml (+S9, 3h treatment + 17h recovery) 0.1-0.5 µg/ml (+S9, 3h treatment + 17h recovery)	Strong increase in the frequency of polyploid cells with and without S9

(+/- S9) = with and without metabolic activation (S9 from Aroclor 1254-treated male rats)

The AMES and COMET screens reveal a signal for bacterial mutagenicity and DNA damaging potential in mammalian cells, respectively. In the 5-strain AMES test, a clear dose-dependent and reproducible mutagenic response was obtained. In the chromosome aberration test with human peripheral blood lymphocytes, a strong increase in the frequencies of polyploid cells (mainly as endoreduplication) was seen in the presence and in the absence of S9.

In conclusion, panobinostat has a clear genotoxic potential in bacterial and eukaryotic systems (mutagenic and endoreduplication inducing effects).

Reproductive Toxicity Studies

Daily oral administration of panobinostat during gestation was associated with the development of embryo-fetal toxicity, including embryo-fetal lethality and an increased risk of skeletal variation and anomalies at maternally toxic doses ≥ 30 mg/kg/day in the rat, and doses ≥ 40 mg/kg/day in

the rabbit, which is approximately 0.18-0.25-fold the expected clinical exposure based upon AUC for a 20 mg dose. No effects on fertility were observed in male rats when panobinostat was administered orally for 4 weeks at doses of 10, 30 and 100 mg/kg. However, male reproductive effects were observed in the testes, epididymides and prostate in 4- and 13-week repeated dose oral toxicity studies in the dog (doses of 1.5 mg/kg and 1.5→1.0 mg/kg; approximately 0.69- and 0.41-fold the expected clinical exposure based upon AUC for a 20 mg dose)

Safety Exposure Multiples

Systemic exposure based on AUC, Cmax and mg/m^2 in cancer patients administered 240 mg using a TIW treatment regimen (20 mg TIW weekly x 4) is similar to exposure in rats and dogs at the maximal tolerated multiple oral doses of 75-100 and 1.5 mg/kg respectively. Safety exposure multiples are shown below in **Table 6**.

Table 6

Animal oral exposure multiples of humans doses of 20 mg

	Animal Dose[a]		Animal Exposure[b]		Safety Margin relative to Human dose of 20 mg (12.3 mg/m^2)[c]		
Species and Study	(mg/kg)	(mg/m^2)[c]	AUC0-24h (ng·h/mL)	Cmax (ng/mL)	Based on human mean AUC[d]	Based on human mean Cmax[e]	Based on body surface area
Dog							
4-week (MTD)	1.5	30	137	52.1	0.69	2.41	2.44
13-Week (MTD)	1.0	20	82.6	23.1	0.41	1.07	1.62
13-Week (NOAEL)	0.15	3	14.9	2.91	0.07	0.13	0.24
39-week (MTD)	1.0	20	81.5	20.1	0.41	0.93	1.62
39-week (NOAEL)	0.15	3	15.25	3.1	0.08	0.14	0.24
Rat							
4-week (MTD)	30	180	187.5	70.1	0.94	3.24	14.64
13-week (MTD)	100	600	441	87.2	2.22	4.04	48.78
26-week (MTD)	75	450	609	204	3.06	9.44	36.58
EFD: Maternal and Embryo-fetal NOAEL	10	60	36.4	4.84	0.18	0.22	4.88
Rabbit							
EFD: Maternal and Embryo-fetal NOAEL	10	120	49.6	15.1	0.25	00.70	9.76

[a]Three oral doses per week administered on Monday, Wednesday and Friday except for EFD studies in which panobinostat was administered daily during gestation.
[b]Average of the mean values from male and female animals, where appropriate
[c]mg/m^2 calculated using a conversion factor of 6 for rat, 12 for the rabbit, 20 for the dog, and 37 for human (assuming a 60 kg human) [U.S. Dept of Health and Human Services, Food and Drug Administration, 2002]
[d]199 ng·h/mL, [e]21.6 ng/mL MTD = Maximum Tolerated Dose, NOAEL = No Observed Adverse Effect Level
Sources: [Study LBH589B2101], [Study LBH589B2102], [Table 2.6.7.7G-Study 0370122-01], [Table 2.6.7.7I-Study 0680133], [Table 2.6.7.7A-Study 0370121-01], [Table 2.6.7.7B-Study 0680019], [Table 2.6.7.7H-Study 0680020-01], [Table 2.6.7.7C-Study 0680134], [Table 2.6.7.13A-Study 0570309-01], [Table 2.6.7.13C-Study 0670512], [Table 2.6.7.13C-Study 0670512]

(Panobinostat Investigator's Brochure 2015)[60]

2.3.3 Adult Clinical Trials

Panobinostat is FDA-approved (in combination with bortezomib and dexamethasone) for use in adult patients with multiple myeloma at a dose of 20 mg p.o. 3x/week x 2 weeks of a 3 week cycle. Panobinostat is available as a capsule. The oral formulation has been administered across multiple

dosing schedules that includes days 1, 3, and 5 every week; or every other week; or days 1 and 4 every week. (The clinical program for the IV formulation is completed with no further company-sponsored studies currently planned.)

2.3.3.1 *Pharmacokinetics*

Absorption

Following oral administration, panobinostat is rapidly absorbed with peak plasma concentration reached within 2 hours; systemic clearance is 33 L/hour and central volume of distribution is 25 liters. Half-lives associated with the distribution, initial elimination and terminal elimination phases were 0.14, 2.3 and 37 hours, respectively. Observed drug accumulation was approximately 1.1 fold with 3 times per week schedule with an effective half-life calculated to be approximately 16 hours. Absolute oral bioavailability is 21%.

Influence of food on panobinostat PK was evaluated in 63 patients with advanced cancer in a randomized 3-way cross-over design under fasting, high fat and standard breakfast conditions. The overall exposure and inter-patient variability in exposure (CV 59%) remained unchanged with or without food, whereas C_{max} was reduced by up to 44% and T_{max} prolonged by 1.5 hours due to food (i.e., under standard or high fat breakfast). Since food did not alter the overall bioavailability in patients, panobinostat can be administered without regard to food[61], although administering in the fasting state to optimize C_{max} may be considered.

Distribution and Protein Binding

Panobinostat is rapidly and extensively distributed to tissues as shown by an approximately 5- fold decrease of the plasma concentration observed within 15 minutes after the end of an IV infusion (V_{ss} 320 L/m^2). In adults, BSA, age and race are statistically significant covariates on the central volume of distribution. This has not yet been studied in children.

The extent of panobinostat binding to human plasma protein was moderate (89.6%) *in vitro* between 0.1 ng/mL and 100 ng/mL panobinostat concentration.

Metabolism and Elimination

Panobinostat is extensively metabolized in patients to over 70 metabolites, all of which are inactive towards the target. As a result, PK was mainly characterized based on the parent compound.

The hydroxamic acid containing side chain is the primary site of panobinostat metabolism, which involves mainly reduction, hydrolysis, and one and two-carbon chain-shortening. Unchanged panobinostat accounts for ≤ 2.5% of the dose in urine and < 3.5% of the dose in feces, the balance representing metabolites. Apparent panobinostat renal clearance (CLR/F) ranged from 2.4 to 5.5 L/h, suggesting that the renal route is a minor elimination pathway of unchanged panobinostat.

Neither panobinostat nor any of the circulating metabolites contributed more than 10% of the total drug-related exposure. Of note, metabolites formed via the primary metabolic pathways were all inactive for DAC inhibitory activity *in vitro*[62].

2.3.3.2 *Adult Phase 1 Studies*

Several dosing schedules using i.v. and oral panobinostat were evaluated with PK assessment. To date, the PK of panobinostat has been characterized in over 700 patients with cancer, of which, over 500 patients used the TIW dosing schedule.

Single Agent Panobinostat

Summary: In clinical trials of panobinostat administered as a single agent to adults with refractory hematologic malignancies[63], C_{max} and AUC increased proportionally with dose over a dose range of 4.8-14.0 mg/m^2. Panobinostat $t_{1/2}$ was 7.8-12 hrs and plasma AUC_{0-24} ranged from 134.9-372.9 h•ng/mL. C_{max} was approximately 20 ng/ml, or about 60 nM[64,65]. Major toxicities included thrombocytopenia, neutropenia, and QTc prolongation that was dose-limiting. Hypokalemia and elevated LFTs (ALT) were reported, as were mild nausea, vomiting and anorexia.

In a Phase 1 trial of single agent panobinostat administered to adults with solid tumors[66], patients received oral panobinostat 3 times per week (M-W-F) at doses ranging from 10-20 mg. No DLT was observed. Most common adverse events included thrombocytopenia, nausea and diarrhea. Mean elimination $t_{1/2}$ was 9-14 hrs.

A trial of panobinostat for refractory hematological malignancies in children is complete (TACL, clinicaltrials.gov ID NCT01321346). No dose-limiting toxicities have been observed at dose levels up to 34 mg/m^2/dose, administered 3 days/week x 4 weeks in children with leukemia[68].

Panobinostat in Combination

Panobinostat has been evaluated in a Phase 1 trial for recurrent adult high-grade glioma (HGG) in combination with bevacizumab. In adults with HGG, the combination of panobinostat with bevacizumab was safe and tolerated at a panobinostat dose of 30 mg PO three times per week, every other week and bevacizumab (10 mg/kg every other week). The main DLT was thrombocytopenia; (one case of grade 3 thrombocytopenia was observed at a dose of 20 mg PO three times per week every week, in combination with bevacizumab)[67].

2.3.3.3 *Phase 2 Studies*

Panobinostat has completed or is undergoing several phase 2 trials in adults with hematological malignancies and solid tumors, alone and in combination with other agents. Notably, a phase 2 trial of panobinostat (30mg PO three times per week) with bevacizumab (10mg/kg every other week) for recurrent adult HGG was completed (clinicaltrials.gov ID NCT00859222). Although well tolerated, the addition of panobinostat to bevacizumab did not significantly improve 6 month progression-free survival compared to bevacizumab monotherapy[74]. Thirty-six clinical studies, including clinical pharmacology, Phase 1 and Phase 2 trials, as well as two randomized Phase 3

studies have either been completed or are ongoing. A total of 2428 adult patients have been enrolled, 235 for the IV formulation and 2193 for the oral formulation, who received at least one dose of panobinostat either as a single agent or in combination with other agents.

Patients were treated with panobinostat either 3 times per week every week (n=666) or 3 times per week every other week (n= 96) in single agent oral panobinostat clinical trials. These patients comprise the pooled safety population experiencing AEs during study treatment. The most frequent non-hematologic toxicities included GI events (diarrhea, nausea, vomiting), mostly of Grade 1-2, in both groups. Blood and lymphatic system disorders were the second most often reported specific system organ class involved, with dose-related thrombocytopenia being the most frequent AE in this category. Fatigue, mostly Grade 1-2, was also common among patients treated for TIW QW and TIW QOW.

AEs regardless of causality for 3 times per week every week dosing were reported in 664 patients, 99.7% of the safety population for this dosing schedule. The most commonly reported AEs across doses were gastrointestinal, including diarrhea (61.0%) and nausea (55.9%). Thrombocytopenia was the third most frequent AE (53.9%) with the highest frequency in the 40 mg dose level (137 patients; 84%). Fatigue also was commonly seen across dose levels in 326 patients (48.9%) overall. Of note, hypothyroidism was reported in 12.9% of patients treated at the dose level of 40 mg, mostly deriving from study [CLBH589E2214] in Hodgkin Lymphoma. (Hodgkin Lymphoma patients are known to have an increased risk for hypothyroidism.)

In studies using a weekly schedule, Grade 3-4 AEs regardless of causality were reported in 534 patients, 80.2% of the safety population. The most commonly reported Grade 3-4 AEs across doses were thrombocytopenia (40.8%), neutropenia (16.7%), anemia (15.5%), fatigue (12.3%) and febrile neutropenia (6.9%). Toxicity appeared dose-related, with more Grade 3-4 hematologic AEs at higher dose levels. The highest incidence of febrile neutropenia was seen at the dose level of 60 mg (27.4%) compared to the other dose levels where the incidence was ≤ 3.7%. This could be because this dose level was only tested in leukemia patients, in whom febrile neutropenia is a common AE. Grade 3-4 thrombocytopenia, Grade 3-4 neutropenia and Grade 3-4 anemia accounted for 75.7%, 82.8% and 52.8% of their respective all grade events. Grade 3-4 diarrhea, Grade 3-4 vomiting and Grade 3-4 nausea accounted for less than 10% of their respective all grades events. For the 3 times per week every other week dosing schedule, AEs regardless of causality were reported in 96 patients, which is 100% of the safety population. The most commonly reported AEs (all grades) across doses were diarrhea (67.7%), nausea (62.5%), fatigue (56.3%), vomiting (43.8%), thrombocytopenia (42.7%), pyrexia (36.5%) and anorexia (34.4%). In the 3 times per week every other week schedule, Grade 3-4 AEs regardless of causality were reported in 81 patients, 84.4% of the safety population. The most commonly reported Grade 3-4 AEs across doses were thrombocytopenia (36.5%), neutropenia (26.0%), fatigue (14.6%), diarrhea (11.5%), anemia (10.4%), and febrile neutropenia (8.3%).

Overall, the most frequent Grade 3-4 AEs regardless of causality for both schedules (3 times per week every week, and 3 times per week every other week) were ascribed to the same SOC, namely blood and lymphatic system disorders. In February 2014, panobinostat was approved by the FDA for patients with recurrent multiple myeloma who have received at least two prior therapy regimens including the proteasome inhibitor, bortezomib and an immunomodulatory agent,

based on a pre-specified subgroup analysis from the PANORAMA-1 trial.

This approval was based on a study of 193 adults with multiple myeloma who had previously received at least 2 treatments, including bortezomib and an immunotherapy drug. In the study, people were randomly assigned to receive a combination of panobinostat, bortezomib, and dexamethasone, or only bortezomib and dexamethasone.

The pre-specified analysis looked specifically at 193 patients from the phase 3 study who were treated with bortezomib and dexamethasone. In this population, the median progression-free survival (PFS) with the panobinostat combination was 10.6 months versus 5.8 months with placebo (HR = 0.52; 95% CI, 0.36-0.76). Additionally, the tumor shrinkage rate with panobinostat was 59% versus 41% with bortezomib and dexamethasone alone[69].

Cardiac Safety

Cardiac safety data for 666 patients treated with oral panobinostat 3 times per week are presented in **Table 7**. All patients underwent intensive pre- and post-dose ECG monitoring. The most common finding is a post-baseline QTcF increase of > 30 and ≤ 60 msec (approximately 22%). No cases of torsades de pointes have been observed. QTcF abnormalities are shown in **Table 7**, for the once per week schedule.

Table 7

QTcF changes in patients receiving oral panobinostat three-times-a-week every-week

QTcF variable	20 mg N=309 n (%)	30 mg N=81 n (%)	40 mg N=163 n (%)	60 mg N=113 n (%)	TOTAL N=666 n (%)
QTcF increase from baseline > 30 and <= 60 ms	47 (15.2)	12 (14.8)	46 (28.2)	38 (33.6)	143 (21.5)
QTcF increase from baseline > 60 ms	7 (2.3)	1 (1.2)	16 (3.7)	13 (11.5)	27 (4.1)
Absolute QTcF > 450 and <= 480 ms	38 (12.5)	10 (12.3)	13 (8.0)	27 (24.5)	88 (13.4)
Absolute QTcF > 480 ms and <= 500 ms	5 (1.6)	1 (1.2)	4 (2.5)	7 (6.2)	17 (2.6)
Absolute QTcF > 500 ms	1 (0.3)	0 (0.0)	10 (0.0)	5 (4.4)	6 (0.9)

N= Number of patients in the group.
n= Number of patients at risk for a designated change with both non-missing baseline and post-baseline values.
Patients are counted only for the worst grade observed post-baseline.

For the every other week schedule, post-baseline increased values of > 30 and ≤ 60 msec were observed (16.7%). Post-baseline increase of > 60 msec was less frequent (4 patients, 4.2%). Absolute QTcF prolongation values of 450 msec to 480 and of > 480 to 500 msec were reported in 9 patients (9.6%) and in 1 patient (1.0%), respectively. Absolute QTcF prolongation above 500 msec was not observed. Of note, the maximum change of QTcF from baseline does not coincide with the peak plasma concentration-time course of panobinostat suggesting a possible delayed effect.

Panobinostat was FDA approved with a Boxed Warning regarding severe and fatal cardiac events, arrhythmias and electrocardiogram (ECG) changes.

Diarrhea

Panobinostat was FDA approved with a Boxed Warning regarding severe diarrhea.
Severe diarrhea occurred in 25% of patients treated with panobinostat. Diarrhea of any grade occurred in 68% of patients treated with panobinostat compared to 42% of patients in the control arm. Diarrhea can occur at any time. Patients must be monitored for hydration status and electrolyte levels, including potassium, magnesium and phosphate.

Panobinostat PK in Combination with Dexamethasone

In clinical studies in multiple myeloma (MM) where panobinostat was given in combination with bortezomib and dexamethasone, the exposure of panobinostat was decreased by 20 to 50% by the concomitant use of dexamethasone (Dex), which is a dose-dependent mild/moderate CYP3A4 inducer. Furthermore, in-silico data showed that the systemic exposure of panobinostat may be decreased by 70% in the presence of strong inducers of CYP3A4. Therefore, it is suggested that the concomitant use of strong CYP3A4 inducers be avoided.

Conclusions on Human Pharmacokinetics and Metabolism

Panobinostat has linear pharmacokinetics at doses of 10-30 mg. Panobinostat can be administered without regard to food. Panobinostat is extensively metabolized and eliminated nearly equally in kidney and liver. Starting dose adjustments are not necessary in patients with severe renal impairment. Starting dose of panobinostat should be reduced in patients with mild and moderate hepatic impairment, and in patients with co-administration of a strong CYP3A inhibitor. Avoid co-administration with medications that are strong CYP3A4 inducers and sensitive CYP2D6 substrates or 2D6 substrates with narrow therapeutic index. Co-administration with medications that are strong CYP3A inducers is to be avoided when combined with panobinostat, bortezomib and dexamethasone[60].

2.4 *Rationale of Proposed Pediatric Study*

We propose the use of panobinostat to assess safety, tolerability, and pharmacokinetics, as well as initial observations of any clinical effect, in pediatric patients with recurrent/progressive diffuse intrinsic pontine glioma (DIPG). Our rationale is based on pre-clinical evidence including the sensitivity of patient-derived DIPG cell lines to histone deacetylase (HDAC) inhibitors in *in vitro* cell culture studies, and tumor inhibitory effect of panobinostat in *in vivo* efficacy studies performed using orthotopic xenograft models of DIPG.

There is extensive preclinical and clinical data on this agent, which is FDA approved in adults with recurrent multiple myeloma at a dose of 20mg 3x/week x 2 weeks of a 3 week cycle[70].

In the present trial, we seek to evaluate this promising drug for use as a single agent in children with recurrent/progressive DIPG. The initial dose level we propose to use is approximately 80%

of the approved adult dose.

Rationale for Amendment 4:
We have enrolled 11 patients to date, 7 so far are evaluable (1 was inevaluable and 3 are still in the DLT period). Two patients enrolled onto dose level 1 and did not experience a DLT. The subsequent 6 patients were enrolled onto dose level 2. One patient experienced a dose limiting toxicity of grade 3 thrombocytopenia, and was dose reduced to level 1. One patient was unevaluable due to disease progression. A second patient experienced dose limiting toxicities of grade 4 thrombocytopenia and grade 4 neutropenia, and was dose reduced to level 1. Given 2 occurrences of DLTs at dose level 2, the subsequent 3 patients have been enrolled onto dose level 1. Should they tolerate dose level 1 (10mg/m2), it will be declared the maximum tolerated dose (MTD) for the 3 weeks on 1 week off schedule.

The recommended phase 2 dose has been identified for children with recurrent/progressive DIPG as 10 mg/m2, and now we will evaluate panobinostat as a single agent in children with DIPG who have not yet progressed in a separate stratum (Stratum 2), using an alternative schedule. Given the limited BBB (blood brain barrier) penetration of panobinostat, it is highly desirable to maximize the dose administered as part of treatment. To improve the tolerability of panobinostat, we propose a new schedule of Monday, Wednesday, Friday dosing every other week. *In vivo* DIPG orthotopic xenograft data show prolonged duration of tumor suppression in mice treated with weekly 20mg/kg systemic panobinostat compared to mice treated 10mg/kg 3 times per week [54], suggesting that higher doses administered less frequently may be more beneficial than lower doses administered more frequently. In addition to the change in dosing schedule, we will be studying DIPG patients prior to progression to avoid confusion between effects of tumor progression and putative toxicity of the agent. Furthermore, our plans for future efficacy studies focus on DIPG patients prior to progression in order to maximize the likelihood of success.

2.5 *Correlative Studies Background*

2.5.1 Rationale for Pharmacokinetic Studies

2.5.1.1 *Hypothesis*

The pharmacokinetics and tolerability of panobinostat in pediatric patients with recurrent/progressive DIPG may differ from prior studies of panobinostat in the adult population as well as in children with leukemia, and may be affected by prior treatment, age, body surface area, steroid use or concomitant medications.

The pharmacokinetics and tolerability of panobinostat in pediatric patients with DIPG who have received standard radiation therapy but have received no other systemic therapy and have not yet progressed may differ from the pharmacokinetics and tolerability of panobinostat in pediatric patients with recurrent/progressive DIPG. More likely, the pharmacokinetics and tolerability may differ with an alternative dosing schedule.

2.5.1.2 *Preclinical and Clinical Data*

Because there are limited pediatric pharmacokinetic (PK) studies of this agent and none in this

population, PK information from this study will be essential for evaluating toxicity and disease response and for refining dosing in future clinical trials of panobinostat. Mandatory pharmacokinetic studies are needed to characterize the full PK profile of panobinostat in this patient population, correlate PK with toxicities, and evaluate effects of concomitant medications such as dexamethasone. Insights into the biologically active dosage will be gained by relating panobinostat systemic exposure (e.g., AUC) to results of pharmacodynamic studies.

In vitro studies indicate that panobinostat is a substrate of CYP3A4, and dexamethasone is a known inducer of CYP3A4. Thus, one might expect lower exposures of panobinostat when administered concurrently with dexamethasone. It will be important to therefore correlate pharmacodynamic effects of panobinostat with PK. The pharmacokinetics of panobinostat may be affected by age, body surface area, steroid use or concomitant medications.

2.5.2 Rationale for Pharmacodynamic Studies

2.5.2.1 *Cell-free DNA in peripheral blood or urine*

Several tumor suppressor genes associated with the malignant phenotype are repressed by epigenetic mechanisms in sporadic cancers. Thus, therapy with DAC inhibitors may alter tumor phenotype and inhibit growth in such tumors. In collaboration with Nada Jabado, who has established a method to detect H3K27 in serum and urine, we will prospectively evaluate this method and evaluate potential changes after treatment with panobinostat.

3 Patient Selection

All subjects must meet the following inclusion and exclusion criteria. No exceptions will be given. Imaging studies to establish eligibility must be done within two weeks, prior to enrollment. Clinical evaluations to establish eligibility must be done within 7 days prior to enrollment.

3.1 *INCLUSION CRITERIA FOR CHILDREN WITH RECURRENT/ PROGRESSIVE DIPG (Stratum 1)*

3.1.1 Diagnosis

Patients with progressive DIPG, as defined by progressive neurologic abnormalities or worsening neurologic status not explained by causes unrelated to tumor progression (e.g., anticonvulsant or corticosteroid toxicity wean, electrolyte disturbances, sepsis, hyperglycemia, etc.), OR an increase in the bi-dimensional measurement, taking as a reference the smallest disease measurement recorded since diagnosis, OR the appearance of a new tumor lesion since diagnosis.

- Please note: patients with a radiographically typical DIPG, defined as a tumor with a pontine epicenter and diffuse involvement of more than 2/3 of the pons, are eligible without histologic confirmation.
- Patients with pontine lesions that do not meet these radiographic criteria will be eligible if there is histologic confirmation of malignant glioma WHO II-IV.

3.1.2 Age

Patients must be ≥ 2 but < 22 years of age at the time of enrollment.

3.1.3 BSA

Patients must have a BSA ≥ 0.80 m^2 for dose 5mg/m^2.
Patients must have a BSA ≥ 0.65 m^2 for doses of 10mg/m^2 - 22 mg/m^2.
Patients must have a BSA ≥ 0.50 m^2 for doses of 28 mg/m^2 - 36 mg/m^2.

3.1.4 Ability to Swallow

Patient must be able to swallow capsules whole.

3.1.5 Performance Status

Karnofsky Performance Scale (KPS for > 16 years of age) or Lansky Performance Score (LPS for ≤ 16 years of age) assessed within 7 days of enrollment must be ≥ 50%. Patients who are unable to walk because of neurologic deficits, but who are up in a wheelchair, will be considered ambulatory for the purpose of assessing the performance score.

3.1.6 Prior Therapy

Patients must have received a minimum of 54 Gy focal irradiation, administered over approximately 42 days, prior to enrollment. Patients must have recovered from the acute treatment-related toxicities (defined as ≤ grade 1) of all prior chemotherapy, immunotherapy, or radiotherapy prior to entering this study.

3.1.6.1 *Myelosuppressive Chemotherapy:*

Patients must have received their last dose of known myelosuppressive anticancer therapy

or immunotherapy at least 21 days prior to enrollment (42 days if prior nitrosourea).

3.1.6.2 *Investigational/Biologic Agent:*

- Biologic or investigational agent (anti-neoplastic):
  Patient must have recovered from any acute toxicity potentially related to the agent and received their last dose of the investigational or biologic agent ≥ 7 days prior to study enrollment.
  - For agents that have known adverse events occurring beyond 7 days after administration, this period must be extended beyond the time during which adverse events are known to occur and discussed with the principal investigator.

- Monoclonal antibody treatment and agents with known prolonged half-lives: At least three half-lives must have elapsed prior to enrollment.
  - Note: A list of the half-lives of commonly used monoclonal antibodies is available on the PBTC webpage under Generic Forms and Templates.

3.1.6.3 *Radiation Therapy*

Patients must have had their last fraction of:

- Craniospinal irradiation or radiation to ≥ 50% of pelvis > 3 months prior to enrollment.
- Focal irradiation to the primary site > 42 days prior to enrollment
- Local palliative irradiation other than previously irradiated primary site (small port) ≥ 14 days

3.1.7 Organ Function

Patients must have adequate organ and marrow function as defined below:

- Absolute neutrophil count ≥ 1,000/mm^3
- Platelets ≥ 100,000/ mm^3 (unsupported, defined as no platelet transfusion within 7 days, and recovery from post-transfusion nadir)
- Hemoglobin ≥ 8 g/dl (may receive transfusions)
- Total bilirubin ≤ 1.5 times institutional upper limit of normal (ULN)
- ALT(SGPT) ≤ 3 x institutional upper limit of normal
- Albumin ≥ 3 g/dl
- Potassium ≥ LLN
- Serum total calcium (correct for serum albumin) or ionized calcium ≥ LLN
- Serum creatinine based on age/gender as noted in **Table 8**. Patients that do not meet the criteria in **Table 8** but have a 24-hour Creatinine Clearance or GFR (radioisotope or iothalamate) ≥ 70 ml/min/1.73 m^2 are eligible.

Table 8

Serum Creatinine for age/gender		
Age	**Maximum Serum Creatinine (mg/dL)**	
	Male	**Female**
2 to < 6 years	0.8	0.8

6 to < 10 years	1	1
10 to < 13 years	1.2	1.2
13 to < 16 years	1.5	1.4
≥ 16 years	1.7	1.4
The threshold creatinine values in this Table were derived from the Schwartz formula for estimating GFR (Schwartz et al. J. Peds, 106:522, 1985) utilizing child length and stature data published by the CDC.		

- Cardiac Function:
  - Left ventricular ejection fraction ≥ 50 by gated radionuclide study OR shortening fraction of ≥ 27% by echocardiogram
  - Patient has no ventricular arrhythmias except for benign premature ventricular contractions.
  - Patient has a QTc interval < 450 ms.

3.1.8 Growth Factors
Patients must be off all colony-forming growth factor(s) for at least 7 days prior to enrollment (i.e. filgrastim, sargramostim or erythropoietin). 14 days must have elapsed if patients received PEG formulations.

3.1.9 Fruit
Patients must agree to avoid grapefruit or grapefruit juice and Seville (sour) oranges during the entire study.

3.1.10 Pregnancy Status
Female patients of childbearing potential must have a negative serum or urine pregnancy test.

3.1.11 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 and for 3 months after the last dose of panobinostat.

3.1.12 Informed Consent
The patient or parent/guardian is able to understand the consent and is willing to sign a written informed consent document according to institutional guidelines.

3.2 *EXCLUSION CRITERIA FOR CHILDREN WITH RECURRENT/ PROGRESSIVE DIPG (Stratum 1)*

3.2.1 Prior Therapy
- Patients who have had > 60 Gy total radiation to the pons (e.g. patients who have received re-irradiation).
- Patients have had prior HDAC, DAC, HSP90 inhibitors for the treatment of their DIPG.
- Patients have had valproic acid within 28 days prior to enrollment.
- Patients have had prior bone marrow transplant.

3.2.2 Neurological Status
Patients have significant acute deterioration in neurologic status in 72 hours prior to enrollment, in the opinion of the treating physician.

3.2.3 Gastrointestinal
- Patients have impairment of GI function or GI disease that may significantly alter the absorption of panobinostat, for example severe inflammatory bowel disease.
- Patients have diarrhea > CTCAE grade 2.

3.2.4 Systemic Illness
Patients have any clinically significant unrelated systemic illness (serious infections or significant cardiac, pulmonary, hepatic or other organ dysfunction), that in the opinion of the investigator would compromise the ability of the patient to tolerate protocol therapy or put them at additional risk for toxicity or would interfere with the study procedures or results.

3.2.5 Other Malignancy
Patients have a history of any other malignancy.

3.2.6 Transfusions
Patients are known to be refractory to red blood cell or platelet transfusions.

3.2.7 Concurrent Therapy
- Patients who are receiving any other anticancer or investigational drug therapy
- Patients who are required to receive any medication which can prolong the QTc interval. Please see **Appendix B: Medications Which May Cause QTc Prolongation**.

3.2.8 Breastfeeding
Female patient is breastfeeding.

3.2.9 Inability to Participate
Patients who in the opinion of the investigator are unwilling or unable to return for required follow-up visits or obtain follow-up studies required to assess toxicity to therapy or to adhere to drug administration plan, other study procedures, and study restrictions

3.3 *INCLUSION CRITERIA FOR CHILDREN WITH NON-PROGRESSED DIPG* or H3K27M+ Thalamic DMG *(Stratum 2)*

3.3.1 Diagnosis
Patients with DIPG or H3K27M+thalamic DMG who have not yet progressed by clinical or radiographic criteria.
- Please note: patients with a radiographically typical DIPG, defined as a tumor with a pontine epicenter and diffuse involvement of more than 2/3 of the pons, are eligible without histologic confirmation.
- Patients with pontine lesions that do not meet these radiographic criteria will be eligible if there is histologic confirmation of malignant glioma WHO II-IV.
- Thalamic Diffuse Midline Glioma patients will be eligible if there is tissue

confirmation of the H3K27M mutation by immunohistochemistry or by gene testing performed in a CLIA certified laboratory of the investigator's choice.

3.3.2 Age
Patients must be ≥ 2 but < 22 years of age at the time of enrollment.

3.3.3 BSA
Patients must have a BSA ≥ 0.80 m^2 for dose 5mg/m^2.
Patients must have a BSA ≥ 0.65 m^2 for doses of 10mg/m^2 - 22 mg/m^2.
Patients must have a BSA ≥ 0.50 m^2 for doses of 28 mg/m^2 - 36 mg/m^2.

3.3.4 Ability to Swallow
Patient must be able to swallow capsules whole.

3.3.5 Performance Status
Karnofsky Performance Scale (KPS for > 16 years of age) or Lansky Performance Score (LPS for ≤ 16 years of age) assessed within 7 days of enrollment must be ≥ 50%. Patients who are unable to walk because of neurologic deficits, but who are up in a wheelchair, will be considered ambulatory for the purpose of assessing the performance score.

3.3.6 Prior Therapy
Patients must have received a minimum of 54 Gy focal irradiation, administered over approximately 42 days, prior to enrollment. Patients must not have received any other prior therapy for treatment of their CNS malignancy besides standard radiation therapy.

Patients must have recovered from the acute treatment-related toxicities (defined as ≤ grade 1) of radiotherapy prior to entering this study.

3.3.6.1 *Radiation Therapy*
Patients must have had their last fraction of focal irradiation to the primary site > 14 days prior to enrollment. Patients must not have received local palliative irradiation or craniospinal irradiation.

3.3.7 Organ Function
Patients must have adequate organ and marrow function as defined below:

- Absolute neutrophil count ≥ 1,000/mm^3
- Platelets ≥ 100,000/ mm^3 (unsupported, defined as no platelet transfusion within 7 days, and recovery from post-transfusion nadir)
- Hemoglobin ≥ 8 g/dl (may receive transfusions)
- Total bilirubin ≤ 1.5 times institutional upper limit of normal (ULN)
- ALT(SGPT) ≤ 3 x institutional upper limit of normal
- Albumin ≥ 3 g/dl
- Potassium ≥ LLN
- Serum total calcium (correct for serum albumin) or ionized calcium ≥ LLN
- Serum creatinine based on age/gender as noted in **Table 9**. Patients that do not meet the criteria in **Table 9** but have a 24-hour Creatinine Clearance or GFR (radioisotope or iothalamate) ≥ 70 ml/min/1.73 m^2 are eligible.

Table 9

Serum Creatinine for age/gender		
Age	**Maximum Serum Creatinine (mg/dL)**	
	Male	**Female**
2 to < 6 years	0.8	0.8
6 to < 10 years	1	1
10 to < 13 years	1.2	1.2
13 to < 16 years	1.5	1.4
≥ 16 years	1.7	1.4
The threshold creatinine values in this Table were derived from the Schwartz formula for estimating GFR (Schwartz et al. J. Peds, 106:522, 1985) utilizing child length and stature data published by the CDC.		

- Cardiac Function:
  - Left ventricular ejection fraction ≥ 50 by gated radionuclide study OR shortening fraction of ≥ 27% by echocardiogram
  - Patient has no ventricular arrhythmias except for benign premature ventricular contractions.
  - Patient has a QTc interval < 450 ms.

3.3.8 Growth Factors
Patients must be off all colony-forming growth factor(s) for at least 7 days prior to enrollment (i.e. filgrastim, sargramostim or erythropoietin). 14 days must have elapsed if patients received PEG formulations.

3.3.9 Fruit
Patients must agree to avoid grapefruit or grapefruit juice and Seville (sour) oranges during the entire study.

3.3.10 Pregnancy Status
Female patients of childbearing potential must have a negative serum or urine pregnancy test.

3.3.11 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 and for 3 months after the last dose of panobinostat.

3.3.12 Informed Consent
The patient or parent/guardian is able to understand the consent and is willing to sign a written informed consent document according to institutional guidelines.

3.4 *EXCLUSION CRITERIA FOR CHILDREN WITH NON-PROGRESSED DIPG* or H3K27M+ Thalamic DMG *(Stratum 2)*

3.4.1 Prior Therapy

- Patients who have had > 60 Gy total radiation to the pons or thalamus (e.g. patients who have received re-irradiation)
- Patients who have had valproic acid within 28 days prior to enrollment.

3.4.2 Neurological Status

Patients have significant acute deterioration in neurologic status in 72 hours prior to enrollment, in the opinion of the treating physician.

3.4.3 Gastrointestinal

- Patients have impairment of GI function or GI disease that may significantly alter the absorption of panobinostat, for example severe inflammatory bowel disease.
- Patients have diarrhea > CTCAE grade 2.

3.4.4 Systemic Illness

Patients have any clinically significant unrelated systemic illness (serious infections or significant cardiac, pulmonary, hepatic or other organ dysfunction), that in the opinion of the investigator would compromise the ability of the patient to tolerate protocol therapy or put them at additional risk for toxicity or would interfere with the study procedures or results.

3.4.5 Other Malignancy

Patients have a history of any other malignancy.

3.4.6 Transfusions

Patients are known to be refractory to red blood cell or platelet transfusions.

3.4.7 Concurrent Therapy

- Patients who are receiving any other anticancer or investigational drug therapy
- Patients who are required to receive any medication which can prolong the QTc interval.

3.4.8 Breastfeeding

Female patient is breastfeeding.

3.4.9 Inability to Participate

Patients who in the opinion of the investigator are unwilling or unable to return for required follow-up visits or obtain follow-up studies required to assess toxicity to therapy or to adhere to drug administration plan, other study procedures, and study restrictions

3.5 *Treatment at Primary Institution*

All experimental protocol therapy should be dispensed and all on treatment imaging studies should be obtained at a PBTC institution. Laboratory studies, excluding pharmacokinetic and biologic assays, may be performed at a CLIA certified laboratory of the investigator's choice. Imaging utilized to determine eligibility may be performed at an outside institution if all required imaging

sequences are included and the study is deemed of adequate quality by the treating team. All required physical examinations, laboratory parameters need to be performed at the primary PBTC institution during the dose finding period of the protocol.

3.6 *Criteria to Start Treatment*

- Subjects must start therapy within seven (7) days of enrollment.
- Laboratory values must be no older than 7 days prior to the start of therapy. If a test that is repeated post enrollment and prior to the start of therapy is outside the limits for eligibility, it must be rechecked within 48 hours prior to the start of therapy. If rechecks are still outside the limits for eligibility, the patient may not receive protocol therapy and will be considered off study
- Cardiac requirements: please see Section 3.3.7.
- If, in the opinion of the treating physician, a patient's neurologic condition significantly worsens within 72 hours prior to treatment, the patient should not start treatment and should be taken Off Study.

4 Registration Procedures

4.1 *CTEP Investigator and Research Associate Registration Procedures*

Food and Drug Administration (FDA) regulations and National Cancer Institute (NCI) policy require all individuals contributing to NCI-sponsored trials to register and renew their registration annually. To register, all individuals must obtain a Cancer Therapy Evaluation Program (CTEP) Identity and Access Management (IAM) account at https://ctepcore.nci.nih.gov/iam . In addition, persons with a registration type of Investigator (IVR), Non-Physician Investigator (NPIVR), or Associate Plus (AP) must complete their annual registration using CTEP's web-based Registration and Credential Repository (RCR) at https://ctepcore.nci.nih.gov/rcr.

RCR utilizes five person registration types.

- IVR — MD, DO, or international equivalent;
- NPIVR — advanced practice providers (e.g., NP or PA) or graduate level researchers (e.g., PhD);
- AP — clinical site staff (e.g., RN or CRA) with data entry access to CTSU applications such as the Roster Update Management System [RUMS], OPEN, Rave, acting as a primary site contact, or with consenting privileges;
- Associate (A) — other clinical site staff involved in the conduct of NCI-sponsored trials; and
- Associate Basic (AB) — individuals (e.g., pharmaceutical company employees) with limited access to NCI-supported systems.

RCR requires the following registration documents:

Documentation Required	IVR	NPIVR	AP	A	AB
FDA Form 1572	✔	✔			
Financial Disclosure Form	✔	✔	✔		
NCI Biosketch (education, training, employment, license, and certification)	✔	✔	✔		
GCP training	✔	✔	✔		
Agent Shipment Form (if applicable)	✔				
CV (optional)	✔	✔	✔		

An active CTEP-IAM user account and appropriate RCR registration is required to access all CTEP and Cancer Trials Support Unit (CTSU) websites and applications. In addition, IVRs and NPIVRs must list all clinical practice sites and Institutional Review Boards (IRBs) covering their practice sites on the FDA Form 1572 in RCR to allow the following:

- Addition to a site roster;
- Assign the treating, credit, consenting, or drug shipment (IVR only) tasks in OPEN;
- Act as the site-protocol Principal Investigator (PI) on the IRB approval; and
- Assign the Clinical Investigator (CI) role on the Delegation of Tasks Log (DTL).

In addition, all investigators acting as the Site-Protocol PI (investigator listed on the IRB approval), consenting/treating/drug shipment investigator in OPEN, or as the CI on the DTL must be rostered at the enrolling site with a participating organization.
Additional information is located on the CTEP website at https://ctep.cancer.gov/investigatorResources/default.htm. For questions, please contact the **RCR Help Desk** by email at [redacted].

4.1.1 CTSU Registration Procedures

This study is supported by the NCI Cancer Trials Support Unit (CTSU). The protocol-specific regulatory documents are found on the PBTC website.

4.1.2 IRB Approval:

Each investigator or group of investigators at a clinical site must obtain IRB approval for this protocol. The IRB approval and supporting documentation will be submitted to the CTSU Regulatory Office by the PBTC Protocol Coordinator. In addition, each IRB approval will be sent to the protocol sponsor, NCI CCR. Submission must be completed before the site will be approved to enroll patients. For PBTC studies, the IRB approval packet will be uploaded to the PBTC. Once all documents are received and confirmed to be accurate and complete, the responsible coordinator will forward the study packet to the CTSU Regulatory Office. Study centers can check the status of their registration packets by querying the Regulatory Support System (RSS) site registration status page of the CTSU members' website by entering credentials at https://www.ctsu.org.

4.1.3 Downloading Site Registration Documents:

Site registration forms may be downloaded directly from the CTSU website.

- Go to https://www.ctsu.org and log in to the members' area using your CTEP-IAM username and password
- Click on the Regulatory tab
- Click on the RSS Browser, then go to "printable CTSU Forms"
- Select and download the two forms noted in Section 4.1.4.

4.1.4 Requirements for PBTC-047 Site Registration:

- CTSU IRB Certification
- CTSU IRB/Regulatory Approval Transmittal Sheet

4.1.5 Submitting Regulatory Documents:

Submit completed forms along with a copy of your IRB Approval and approved Informed Consent to the PBTC via the PBTC document upload system. Once all documents are received, the responsible Protocol Coordinator listed on the study cover page will submit the regulatory documents to the CTSU Regulatory Office, where they will be entered and tracked in the CTSU RSS.

In addition, the Site-Protocol Principal Investigator (PI) (i.e. the investigator on the IRB/REB approval) must meet the following criteria in order for the processing of the IRB approval record to be completed:

- Holds an active CTEP status;
- Rostered at the site on the IRB approval and on at least one participating roster;
- Includes the IRB number of the IRB providing approval in the Form FDA 1572 in the RCR profile; and
- Holds the appropriate CTEP registration type for the protocol.

Additional Requirements

Additional requirements to obtain an approved site registration status include:

- An active Federal Wide Assurance (FWA) number;
- An active roster affiliation with PBTC; and
- Compliance with all protocol-specific requirements (PSRs)

4.1.6 Checking Your Site's Registration Status:

Site registration status may be verified on the CTSU members' website.

- Click on *Regulatory* at the top of the screen;
- Click on *Site Registration; and*
- Enter the sites 5-character CTEP Institution Code and click on Go.
    - Additional filters are available to sort by Protocol, Registration Status, Protocol Status, and/or IRB Type.

Note: The status shown only reflects institutional compliance with site registration requirements as outlined within the protocol. It does not reflect compliance with protocol requirements for individuals participating on the protocol or the enrolling investigator's status with NCI or their affiliated networks.

4.2 *General Guidelines*

4.2.1 Prior to Consent

Prior to consenting a patient, site staff must verify a slot is available by checking either the PBTC Protocol Status web page or using the CTSU OPEN Slot Reservation System.

4.3 *Enrollment Procedures*

The Oncology Patient Enrollment Network (OPEN) is a web-based registration system available on a 24/7 basis. OPEN is integrated with CTSU regulatory and roster data and with the PBTC registration/randomization systems for retrieval of patient registration/randomization assignment. OPEN will populate the patient enrollment data in NCI's clinical data management system, Medidata Rave.

Requirements for OPEN access:

- A valid CTEP-IAM account;
- To perform enrollments or request slot reservations: Must be on an PBTC roster with the role of Registrar. Registrars must hold a minimum of an Associate Plus (AP) registration type;
- The registrars must hold the OPEN Registrar task on the DTL for the site; and
- Have an approved site registration for the protocol prior to patient enrollment.

To assign an Investigator (IVR) or Non-Physician Investigator (NPIVR) as the treating, crediting, consenting, drug shipment (IVR only), or receiving investigator for a patient transfer in OPEN, the IVR or NPIVR must list the IRB number used on the site's IRB approval on their Form FDA 1572 in RCR. The IVR or NPIVR must be assigned the appropriate OPEN-related tasks on the DTL.

Prior to accessing OPEN, site staff should verify the following:

- Patient has met all eligibility criteria within the protocol stated timeframes; and
- All patients have signed an appropriate consent form and HIPAA authorization form (if applicable).

Note: The OPEN system will provide the site with a printable confirmation of registration and treatment information. You may print this confirmation for your records.

Access OPEN at https://open.ctsu.org or from the OPEN link on the CTSU members' website. Further instructional information is in the OPEN section of the CTSU website at https://www.ctsu.org or https://open.ctsu.org. For any additional questions, contact the CTSU Help Desk at 1-888-823-5923 or [redacted].

Patient enrollment for this study will be facilitated using the Slot Reservation System in conjunction with the registration system in OPEN. Prior to discussing protocol entry with the patient, all site staff must use the CTSU OPEN Slot Reservation System to ensure that a slot on the protocol is available to the patient. Once a slot reservation confirmation is obtained, site staff may then proceed to enroll the patient to this study.

5 Treatment Plan

Treatment may 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 tumor.

5.1 *Agent Administration*

Patients with recurrent/progressive DIPG will be enrolled at the time of progression in Stratum 1. Therapy with panobinostat will be administered every other day, 3 times/ week, p.o. preferably on a Monday/Wednesday/Friday schedule for three weeks, followed by one week off of therapy. Three weeks of therapy plus the one week rest period (total 4 weeks) will constitute one course. Treatment will continue for up to 26 courses (approximately 2 years) barring progressive disease or unacceptable toxicity.

Patients with DIPG or H3K27M+ Thalamic DMG who have not progressed will be enrolled after receiving standard radiation therapy in stratum 2 and cannot begin treatment until at least 14 days after completion of radiation. Therapy with panobinostat will be administered every other day, 3 times/ week, every other week. **A minimum of 6 days without drug is required between treatment weeks.** Four weeks will constitute one course. Treatment will continue for up to 26 courses (approximately 2 years) barring progressive disease or unacceptable toxicity.

5.1.1 Panobinostat

Panobinostat will be provided by Secura Bio Inc. Oral panobinostat will be supplied as 5 mg, 10 mg, 15 mg orange colored, and/or 20 mg reddish colored opaque, hard gelatin capsules.

Subjects will be assigned a dose level at the time of enrollment. See Section 5.1.6 below for details of dose escalation schedule. See Section 6 for dose modifications.

Dosing should be adjusted based on BSA calculated at the beginning of each course of therapy. The dose prescribed should be rounded to the nearest deliverable dose based on the BSA adjustment and the available pill sizes. Dosing Tables which reflect this approach are available in **Appendix D: Dosing Tables**. Patients will be provided with a Medication Diary for panobinostat, instructed in its use, and asked to bring the diary as well as the remaining pill bottles with them to each appointment. The Patient Diary is available in

Appendix E: Patient Diary.
Patients are encouraged to take their dose of panobinostat at the same time each day, preferably in the morning. Each dose of panobinostat should be taken with a 4 oz / 120 ml glass of water. Drug must be taken on an empty stomach (either 1 hour before or after meals) on Course 1, Days 1 and 3, and may be taken with or without food for the remaining doses. Patients should be instructed to swallow the capsules whole and not chew them. Patients must avoid grapefruit or grapefruit juice and Seville (sour) oranges during the entire study.

If the patient forgets to take his/her dose during the morning on a scheduled treatment day, then he/she should take panobinostat on that same day within 12 hours after the missed dose if possible. After more than 12 hours, that day's dose should be withheld, and the patient should wait to take panobinostat until the next scheduled treatment day (i.e., patients should be instructed not to try to make-up the missed dose after 12 hours). The patient should then continue treatment with the original dosing schedule. If vomiting occurs within 15 minutes, the dose should be repeated. If a dose is missed/ unable to be given, record on the patient diary and continue counting the days of the course, with treatment administered on originally scheduled days.

5.1.2 Criteria for Starting Subsequent Courses

A course may be repeated every 28 days if the patient has at least stable disease, has not met any off treatment criteria (Section 5.3.2), has again met laboratory parameters as defined in Section 3.1.7, and any significant toxicity has resolved to acceptable parameters as indicated in Section 6.

5.1.3 Dose-Limiting Toxicity

Management and dose modifications associated with adverse events are outlined in Section 6. DLT will be defined as any of the events listed in this section that are at least possibly related to panobinostat that occur during the dose-finding period regardless of expectedness. The dose-finding period is defined as the first course of therapy in both strata.

Management and dose modifications for toxicities which occur outside of the dose-finding period should also follow Section 6; however, these will not be considered dose limiting for the purpose of dose escalation.

5.1.4 Definitions of Dose-Limiting Toxicities (DLTs):

Patients who miss more than 2 doses due to toxicity will be considered to have experienced a DLT.

5.1.4.1 *Non-hematologic dose limiting toxicity is defined as:*

- Any Grade 3 or greater non-hematologic toxicities with the specific exclusion of the following:
  - Grade 3 nausea/vomiting that is responsive to antiemetics, and that resolves to ≤ Grade 2 within 5 days
  - Grade 3 electrolyte abnormality that resolves to ≤ Grade 2 within 5 days
  - Grade 3 rash that is not considered medically significant or intolerable by the

patient
- Grade 3 diarrhea that resolves to ≤ Grade 1 with optimal use of anti-diarrheal medication
- Grade 3 fever that resolves to ≤ Grade 2 within 5 days
- Grade 3 infection that resolves to ≤ Grade 2 within 5 days

- Any Grade 2 non-hematological toxicity that persists for more than 7 days and is considered sufficiently medically significant or sufficiently intolerable by patients as to warrant treatment interruption and/or dose reduction will be considered dose limiting.
- Any panobinostat-related non-hematological toxicity that results in a delay of treatment > 14 days between treatment courses

5.1.4.2 *Hematologic dose limiting toxicity is defined as:*

- Grade 4 thrombocytopenia (platelet count < 25,000/μL)
- Grade 3 thrombocytopenia with bleeding
- Grade 3 thrombocytopenia (platelet count ≥ 25,000 but <50,000/μL) that occurs twice within a treatment course will be considered a DLT. (Note: platelet transfusions are recommended for platelet counts <50,000/μL and transfusion should target platelet corrections to >100,000 /μL)
- Myelosuppression that causes greater than a 14-day delay between treatment courses
- Grade 4 neutropenia
- Grade 3 or 4 febrile neutropenia

5.1.4.3 *Follow-up for toxicities:*

Patients whose treatment is interrupted or permanently discontinued due to an adverse event or abnormal laboratory value must be followed until resolution or stabilization of the event, or initiation of another antitumor treatment, or initiation of hospice/symptomatic care only, whichever comes first.

All patients will be followed for adverse events and serious adverse events for at least 30 days following the last dose of oral panobinostat, or upon initiation of another antitumor treatment.

5.1.5 Dose-finding Period

The dose finding period begins with the initial dose of panobinostat and ends on the last day of course 1 (approximately 4 weeks). Should there be a delay starting the subsequent course, dose finding will complete on the start date of the subsequent course.

5.1.6 Dose Escalation Schema

Enrollment will commence at dose level 1 i.e. 10mg/m^2. The dose finding will be governed by a two stage continual reassessment method (CRM), as detailed in Section 13 of this protocol. No intra-patient dose escalations will be allowed.

Table 10 below provides the dose escalation schedule proposed for Stratum 1. While the starting dose level is lower than what has been tolerated in the pediatric Phase 1 study in hematologic malignancies, it reflects the dose levels agreed upon by Novartis. Also note that the dose increments are higher than the conventional 30% in the lower doses. This was done in order to avoid overlaps in the deliverable doses due to pill size limitations as applied to BSA based dosing. Please see Section 13.1.1 for further details.

Table 10: Proposed dose escalation schedule for panobinostat in children with recurrent/progressive DIPG (Stratum 1)

Dose level #	***panobinostat oral dose (mg)***	***Minimum BSA Restriction***
*0**	5 mg/m^2/day MWF, three weeks on, one week off (1 course = 28 days)	Patients must have a BSA ≥ 0.80 m^2
1 (starting dose level)	10 mg/m^2/day MWF, three weeks on, one week off, (1 course = 28 days)	Patients must have a BSA ≥ 0.65 m^2
2	16 mg/m^2/day MWF, three weeks on, one week off, (1 course = 28 days)	Patients must have a BSA ≥ 0.65 m^2
3	22 mg/m^2/day MWF, three weeks on, one week off, (1 course = 28 days)	Patients must have a BSA ≥ 0.65 m^2
4	28 mg/m^2/day MWF, three weeks on, one week off, (1 course = 28 days)	Patients must have a BSA ≥ 0.50 m^2
5	36 mg/m^2/day MWF, three weeks on, one week off, (1 course = 28 days)	Patients must have a BSA ≥ 0.50 m^2
	Panobinostat will be administered as a single agent *** Dose level 0 represents a potential treatment dose for patients requiring a dose reduction from dose level 1 and may be used as a contingency dose level if the starting dose level of panobinostat is not tolerated in the initial cohort.**	

Upon completion of Stratum 1, patients with DIPG who have received adequate radiation therapy but have not yet progressed will be enrolled in Stratum 2. Panobinostat will be administered every other day, 3 times/week, every other week p.o. preferably on a Monday/Wednesday/Friday schedule. Total 4 weeks will constitute one course. Treatment will continue for up to two years (26 courses) unless the patient experiences progressive disease, unacceptable toxicity or any of the off-treatment criteria.

The starting dose (dose level 1) for Stratum 2 will be 1 dose level above the highest safe dose for the '3-weeks on, 1-week off' schedule as determined in Stratum 1. The rationale is that 1 week on

1 week off schedule represents a reduction in the amount of drug patients will receive per course and thus a dose level that is safe for '3-weeks on, 1-week off' schedule should also be safe for the every other week schedule.. The current expectation is that 10mg/m^2 will be the MTD for the '3-weeks on, 1-week off' schedule and thus we expect to start exploring the 'every other week' dosing schedule at 16 mg/m^2/day. However, if 10mg/m^2 dose level is determined to be unsafe in the '3-weeks on, 1-week off' schedule we will abandon that schedule and initiate dose escalation for Stratum 2 at 10mg/m^2. The table below lists the proposed dose levels to be studied:

Table 11

Proposed dose escalation schedule for panobinostat in children with non-progressed DIPG or H3K27M+ Thalamic DMG (Stratum 2)

Dose level #	***panobinostat oral dose (mg)***	***Minimum BSA Restriction***
*-1**	5 mg/m^2/day MWF, every other week (1 course = 28 days)	Patients must have a BSA ≥ 0.80 m^2
*0**	10 mg/m^2/day MWF, every other week (1 course = 28 days)	Patients must have a BSA ≥ 0.65 m^2
1	16 mg/m^2/day MWF, every other week (1 course = 28 days)	Patients must have a BSA ≥ 0.65 m^2
2	22 mg/m^2/day MWF, every other week (1 course = 28 days)	Patients must have a BSA ≥ 0.65 m^2
3	28 mg/m^2/day MWF, every other week (1 course = 28 days)	Patients must have a BSA ≥ 0.50 m^2
4	36 mg/m^2/day MWF, every other week (1 course = 28 days)	Patients must have a BSA ≥ 0.50 m^2
	Panobinostat will be administered as a single agent *** Dose levels 0 and -1 represent potential treatment doses for patients requiring a dose reduction from dose level 1 and may be used as a contingency dose level if the starting dose level of panobinostat is not tolerated in the initial cohort.**	

If patients are unable to tolerate the protocol-specified dosing schedule, dose adjustments are permitted in order to keep the patient on study drug. If administration of panobinostat must be interrupted because of unacceptable toxicity, drug dosing will be interrupted or modified according to Sections 6.2 and 6.3. The first course (day of first dose until initiation of Course 2) will be the DLT observation period.

5.2 *Concomitant Medications and Supportive Care Guidelines*

5.2.1 Potential QTc prolonging medications

Concomitant medications which can prolong the QTc interval should not be used. Please see **Appendix B: Medications Which May Cause QTc Prolongation,** for a partial list of medications with the potential to prolong the QTc interval. Providers should consult a frequently-updated drug information reference for a complete list.

5.2.2 CYP3A4 and CYP2D6

Co-administration with medications that are strong CYP3A4 inducers should be avoided. Co-administration with medications that are strong CYP3A4 inhibitors and sensitive CYP2D6 substrates is feasible when medically necessary and with close monitoring. Please see **Appendix** C: Partial List of **Common Moderate and Strong Inhibitors of CYP3A4, and Common Sensitive CYP2D6 Substrates**. Providers should consult a frequently-updated drug information reference for a complete list.

5.2.3 Steroids

Corticosteroids should be used at the lowest dose to control symptoms of edema and mass effect, and discontinued, if possible. Use of corticosteroids should be recorded in the PBTC Rave database.

5.2.4 Anticonvulsants

Anticonvulsants drugs should be used, if indicated. Use of anticonvulsants should be recorded in the PBTC Rave database. Note: Use of valproic acid or enzyme inducing anti-convulsants is prohibited on this trial. Note that levetiractam (Keppra) may cause prolongation of the QTc and patients who must remain on Keppra should be closely monitored.

5.2.5 Growth Factors

Routine use of growth factors (i.e. G-CSF, GM-CSF and erythropoietin) is not permitted. However, therapeutic use of G-CSF or GM-CSF in patients with serious neutropenic conditions, such as sepsis, may be considered at the investigator's discretion. Use of growth factors should be recorded in the PBTC Rave database.

5.2.6 Anti-emetics

The use of anti-emetics will be at the investigator's discretion. Use of anti-emetics should be recorded in the PBTC Rave database. Steroids should not be used as anti-emetics when possible.

5.2.7 Febrile neutropenia

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

include laboratory testing, blood and urine cultures, and institution of broad spectrum antibiotics.

5.2.8 Pneumocystis jiroveci pneumonia (PJP) prophylaxis

The use of medication (e.g., Bactrim) for PJP prophylaxis in patients on chronic steroids is recommended but is at the investigator's discretion.

5.2.9 Neurosurgical or other surgical procedures

If a neurosurgical procedure or other surgical procedure is required for a reason other than tumor progression (i.e. the onset of hydrocephalus), these procedures should be documented, but will not constitute criteria for declaring the patient "off therapy". Panobinostat should be held until the patient is clinically stable and has recovered from the acute effects of surgery.

5.2.10 Diarrhea

Patients should be advised to drink plenty of water or rehydration fluids to avoid dehydration if diarrhea occurs. Should diarrhea occur, investigators should ensure that patients have loperamide on hand and follow the loperamide dosing guidelines in **Table 12** below, at first onset of the symptom. Dehydration prevention and correction of electrolyte disturbances should be practiced as per institution's SOP. Stool count and consistency should be noted in the patient diary if treatment for diarrhea is instituted.

Refer to **Table 13** for details of panobinostat dose reduction, and **Table 12** below for details of suggested loperamide dosing based on patients body weight.

Table 12

Weight Specific Guidelines for Therapeutic Use of Loperamide			
Weight (kg)	Initial (Loading) Loperamide dose (mg)	Subsequent daytime loperamide dose	Subsequent nighttime loperamide dose
8-10	1	0.5mg q 3h	0.75mg q 4h
10-20	1	1mg q 3h	1mg q 4h
20-30	2	1mg q 3h	2mg q 4h
30-42	2	1mg q 2h	2mg q4h
42	4	2mg q 2h	4mg q 4h

5.3 *Duration of Therapy*

In the absence of treatment delays due to adverse event(s) or disease progression, treatment may continue for 26 courses (approximately 2 years) or until one of the Off Treatment Criteria applies as noted in Section 5.3.2.

5.3.1 On Study Data Submission Schedule

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 Rave database. See the Required Data

and Timetable for Submission form located on the PBTC-047 Protocol webpage for the schedule. For assistance, contact the PBTC Protocol Coordinator listed on the cover page. An optional roadmap is located on the PBTC-047 Protocol Webpage.

5.3.2 Off Treatment Criteria

At the discontinuation of treatment, the "Off Treatment Date" is to be recorded in the eCRF and is to be consistent with the reason given for going off treatment. The "Last Treatment Date" is defined as the last date that the patient received protocol based therapy. Date of "off treatment" must be the greatest of the date of last treatment, date of procedure, date of patient assessment, 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.
Patients will be considered Off Treatment for the following reasons:

- Development of unacceptable toxicity as outlined in Section 5.1.3. See Section 7 for specific adverse event reporting requirements.
- Progressive disease (PD) as described in Section 11.2.4.
- Development of a medical or psychiatric illness or social issue that in the investigator's judgment renders the patient incapable of further therapy on this protocol or the treating physician determines continuation on this study is not in the patient's best interest.
- The patient, parent or legal guardian refuses further treatment on this protocol. In this case the investigator should clarify if the family also wishes to withdraw consent for continued participation for data collection purposes.
- Completion of all protocol defined treatment
- Pregnancy
- Non-compliance that in the opinion of the investigator does not allow for ongoing participation.

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

5.3.3 Data Submission Schedule for Patients Off-Treatment

Patients will be followed initially for the resolution of all toxicities considered at least possibly related to panobinostat occurring while on treatment and for 30 days after the last administration of study drug. Patients will continue to 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. Once patients are off-treatment and treatment-related toxicities have resolved or another treatment started, follow up during this 3-year period may be done electronically or by phone. Data should be updated quarterly in the PBTC Rave database.

5.3.4 Criteria for Removal from Study

The date and reason for the patient coming off study must be documented in the eCRF and the OBDMC must be notified according to standard reporting guidelines (see Sections 7 for adverse events, Section 5.3.5 for Data submission for Off Study, Section 12 for Data Reporting, and the Required Data and Timetable Submission from located on the PBTC-047 protocol webpage).

- Patient determined to be ineligible.
- Parent, patient, or guardian withdraws consent for continued participation.
- Patient death while on study. The IRB, Study Chair and OBDMC must be notified as per Section 7.3.
- Patients removed from treatment for reasons other than withdrawal of consent or Death will be followed until three years have elapsed from the date of initiation of protocol therapy on this study.

5.3.5 Data Submission for Patients Off-Study

No data will be collected documenting treatment or reporting events or disease status that occur 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 an IND (see **Section 7**).

6 Dosing Delays/ Dose Modification

6.1 *Notification of Study Chair*

The study chair or co-chair must be notified of any dosage modifications, prior to the implementation of the dose modification

6.2 *Hematologic and Non-Hematologic Adverse Events and Management*

The criteria for dose modifications for study drug-related toxicity are detailed in **Table 13**.

Dose Modification for Hematological Toxicity

If a patient experiences dose-limiting hematological toxicity as outlined in Section 5.1.4.2 (i.e. grade 4 thrombocytopenia [$< 25 \times 10^9$/L] or grade 3 thrombocytopenia [< 50 and $\geq 25 \times 10^9$/L] with bleeding, or grade 3 twice within 1 treatment cycle or grade 4, or grade 3 or 4 febrile neutropenia) treatment will be withheld. Platelets should be transfused to a count of $> 100 \times 10^9$/L (i.e. a post-transfusion platelet count should be obtained). Counts should then be checked every 3-4 days (and when medically indicated) during this time.

If the toxicity resolves to meet on study parameters within 14 days of drug discontinuation, provided there is no current active bleeding in the case of dose-limiting thrombocytopenia, the patient may resume therapy at one dose level lower. Patients who are dose-reduced for toxicity will not have their dose re-escalated.

If toxicity does not resolve to meet on study parameters within 14 days of drug discontinuation, the patient must be removed from protocol therapy.

Only one dose-reduction per patient is allowed. If any dose-limiting toxicity occurs in a patient already dose-reduced for toxicity, the patient must be removed from protocol therapy.

Table 13

Criteria for dosing delays, dose-reductions, and re-initiation of treatment due to study drug-related non-hematological toxicity (excluding QT prolongation)

Worst Toxicity **CTCAE Grade* unless otherwise specified (Value)**		**Dose Modification Guidelines** **At any time during a course of therapy (including intended day of dosing) Note:** If dose limiting toxicity does not resolve after holding drug for 14 days, patient will be removed from treatment
NON-HEMATOLOGICAL TOXICITIES		
CARDIAC		
Cardiac - Prolonged QT interval**		Please refer to Section 6.3.
GASTROINTESTINAL		
Diarrhea	Grade 2 (4-6 stools/day over baseline, etc.) despite the use of optimal antidiarrheal medications	None. If unacceptable to patient or medically concerning, then hold until recovery to ≤ grade 1, or baseline, up to 14 days, then restart original schedule at unchanged dose level
	Grade 3 (≥ 7 stools/day over baseline, etc.) despite the use of optimal antidiarrheal medications	Temporarily discontinue panobinostat dosing until resolved to ≤ grade 1, or baseline, then restart original schedule reduced by one dose level
	Grade 4 (life-threatening consequences, hemodynamic collapse, etc.) despite the use of optimal antidiarrheal medications	Discontinue panobinostat dosing
Vomiting/Nausea***	Grade 1, 2 not requiring treatment or controlled using standard anti-emetics	Maintain dose level
	Grade 3 that resolves to ≤ grade 2 with standard emetics within 5 days	Maintain dose level
	Grade 3 or 4 vomiting or Grade 3 nausea that cannot be controlled or does not resolve to ≤ grade 2 within 5 days despite the use of standard anti-emetics	Temporarily discontinue panobinostat dosing until resolved to ≤ grade 1, or baseline, then restart panobinostat reduced by one dose level
FATIGUE		
Fatigue	Grade 3	Temporarily discontinue panobinostat dosing until resolved to ≤ grade 2, or baseline, then: • If resolved within 7 days after suspending panobinostat, then restart panobinostat at an unchanged dose level • If resolved in more than 7 days after suspending panobinostat, then restart panobinostat reduced by one dose level

Worst Toxicity **CTCAE Grade* unless otherwise specified (Value)**		**Dose Modification Guidelines** **At any time during a course of therapy (including intended day of dosing) Note:** If dose limiting toxicity does not resolve after holding drug for 14 days, patient will be removed from treatment
NON-HEMATOLOGICAL TOXICITIES		
	Grade 4	Temporarily discontinue panobinostat dosing until resolved to ≤ grade 2, or baseline, then: • restart panobinostat reduced by one dose level
HEPATIC		
Total Bilirubin	Grade 3 or 4	Temporarily discontinue panobinostat dosing until resolved to ≤ grade 2, or baseline, then restart panobinostat reduced by one dose level
Note: If Grade 3 or Grade 4 hyperbilirubinemia is due to the indirect component only, and hemolysis as the etiology has been ruled out as per institutional guidelines (e.g., review of peripheral blood smear and haptoglobin determination), then reduction of one dose level and continuation of treatment is at the discretion of the Investigator.		
AST/SGOT, ALT/SGPT	> 5-10 x ULN	Temporarily discontinue panobinostat dosing until resolved to ≤ grade 1 or baseline, then: • If resolved within 7 days, then: • restart panobinostat at unchanged dose level • If resolved in more than 7 days, then reduce panobinostat by one dose level
	> 10 x ULN	Temporarily discontinue panobinostat dosing until resolved to ≤ grade 1, or baseline, then: • restart panobinostat reduced by one dose level
All dose modifications should be based on the worst preceding toxicity.		
* Common Terminology Criteria for Adverse Events (CTCAE Version 4.0 or 5.0; see Section 7.2)		
** It is critical that electrolyte abnormalities be followed closely and corrected prior to dosing		
*** See also concomitant medication Section 5.2.		

6.3 *Dose Modifications for Prolonged QTc*

All cardiac events should be treated according to the local standard of care and referred to a cardiologist if clinically indicated. The localized readings of ECGs will use the Fridericia correction for QTC interval assessment: QTcF. Any final decisions concerning dose modifications or permanently discontinuing the patient from study drug due to QTcF prolongation will be based on the Investigator's clinical assessment. Any plan to deviate from these guidelines must be previously discussed and agreed upon with the PI.

Patients must have QTcF < 450 msec to be eligible for the trial. If QTcF is ≥ 450 msec or above 60 msec from pre-treatment ECG, correct any electrolyte abnormalities (hypokalemia, hypomagnesemia, hypocalcemia), and conduct triplicate ECGs (5 minutes apart) and calculate average QTcF. If the average QTcF from the triplicate ECGs remains ≥ 450ms, patient must not be dosed. Pre-dose ECG monitoring is mandatory on days 1 and 5 of course 1, and day 1 of courses 2 through 4. Post dose ECG (3 hours post dosing) is mandatory only on days 1and 5 of course 1. On day 5 of course 1, ECG may be performed at a local institution but results must be faxed to treating institution within the same day.

If a pre-treatment or post-dose ECG shows a QTcF ≥ 500ms, patient is not eligible for further treatment. If any of these mandatory ECGs show QTcF ≥ 450 msec or 60 msec above baseline, dosing should be held and the above specified measures including follow-up triplicate ECGs and correction of electrolyte abnormalities should be performed. If QTcF abnormality resolves, patients should resume treatment and have QTcF checked pre-dose on day 1 of each course for at least the next 3 subsequent courses. Additional QTcF monitoring should be performed if clinically indicated. If a patient cannot be dosed due to prolonged QTcF for more than 7 days since last dose, patient must be discontinued from study treatment.

Table 14

Dose Reductions for QTc Prolongation

Time Point	**Average QTcF***	**Action**
Screening	≥ 450 msec	Patient is not eligible to enroll
pre-dose course 1 day 1	≥ 470 msec	Delay treatment Correct any electrolyte abnormal values ** and repeat ECG, if the average QTcF ≥ 470 msec, do not dose
	Above 500 msec	Patient is not eligible to receive any treatment
Pre-dose course 1 day 5 Pre-dose day 1 of subsequent courses	≥ 450 msec or above 60 msec from baseline for any pre-dose ECG after the patient has commenced treatment***	Omit dose If unresolved within 7 days, discontinue treatment If resolved within 7 days, resume treatment at prior dose for initial occurrence or at reduced dose if recurrent
	Above 500 msec	Permanently discontinue treatment
*QTcF: Heart rate corrected QT interval using the Fredericia formula: QTc=QT/RR0.33 **: serum potassium, magnesium, calcium, ***If a single pre-dose QTcF is ≥ 450 msec or 60 msec from baseline, subsequent ECGs should be performed in triplicate		

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 7.2) 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. The coding of attribution in the PBTC Rave database pertains to adverse events related to panobinostat.

- *Baseline Abnormalities*

Any baseline (pretreatment) abnormalities observed during the initial physical examination should be recorded in the PBTC Rave database.

- *Treatment or within 30 days of treatment*

Only record adverse events grades 1 and 2 if the attribution is at least possibly related to panobinostat. Record all adverse events grades 3 through 4 and deaths), regardless of attribution on the electronic case report forms.

7.1 *Adverse Events and Potential Risks for Panobinostat*

7.1.1 **Very Common** adverse events occurring in **≥ 10%** of subjects treated with panobinostat include, but may not be limited to:

AE Category	Event Descriptions Included:
Infections and infestations	Upper respiratory tract infection, Pneumonia
Metabolism and nutrition disorders	Decreased appetite
Psychiatric disorders	Insomnia
Nervous system disorders	Dizziness, Headache
Cardiac disorders	Electrocardiographic abnormalities such as ST-segment depression and T-wave abnormalities
Vascular disorders	Hypotension
Respiratory, thoracic and mediastinal disorders	Cough, Dyspnea
Gastrointestinal disorders	Diarrhea, Nausea, Vomiting, Abdominal pain, Dyspepsia
General disorders and administration site conditions	Fatigue, Edema peripheral, Pyrexia, Asthenia
Investigations	Weight decreased
Hematologic	Thrombocytopenia, Anemia, Leukopenia, Neutropenia, Lymphopenia
Biochemistry	Blood creatinine increased, Hypokalemia, Hypophosphatemia, Hyponatremia, Hyperbilirubinemia, SGPT Alanine amino

	transaminase (ALT) increased, SGOT Aspartate amino transaminase (AST) increased
Cardiac disorders	Arrhythmias

7.1.2 **Common** adverse events occurring in **1% to 9%** of subjects treated with panobinostat:

AE Category	Event Descriptions Included:
Infections and infestations	Septic shock, urinary tract infection, viral infection, oral herpes, clostridium difficile colitis, otitis media, cellulitis, sepsis, gastroenteritis, lower respiratory tract infection, candidiasis
Endocrine disorders	Hypothyroidism
Metabolism and nutrition disorders	Hyperglycemia, dehydration, hypoalbuminemia, fluid retention, hyperuricemia, hypocalcemia, hypomagnesemia
Nervous system disorders	Intracranial hemorrhage, syncope, tremor, dysgeusia
Eye disorders	Conjunctival hemorrhage
Cardiac disorders	Bradycardia, atrial fibrillation, sinus tachycardia, tachycardia, palpitations; cardiac ischemic events
Vascular disorders	Hypertension, hematoma, orthostatic hypotension
Respiratory, thoracic and mediastinal disorders	Respiratory failure, rales, wheezing, epistaxis
Gastrointestinal disorders	Gastrointestinal hemorrhage, hematochezia, gastritis, cheilitis, abdominal distension, dry mouth, flatulence
Hepatobiliary disorders	Hepatic function abnormal
Skin and subcutaneous disorders	Skin lesions, rash, erythema
Musculoskeletal and connective tissue disorders	Joint swelling
Renal and urinary disorders	Renal failure, hematuria, urinary incontinence
General disorders and administration site conditions	Chills, malaise
Investigations	Blood urea increased, glomerular filtration rate decreased, blood alkaline phosphatase increased, electrocardiogram QT prolonged

7.1.3 Uncommon adverse events occurring in **< 1%** of subjects treated with panobinostat**:**

AE Category	Event Descriptions Included:
Infections and infestations	Pneumonia fungal, hepatitis B, aspergillosis; infection-related deaths, primarily pneumonia and/or sepsis that could be associated with respiratory or multi organ failure
Cardiac disorders	Myocardial infarction
Vascular disorders	Hemorrhagic shock; gastrointestinal and pulmonary hemorrhage-related death
Respiratory, thoracic and mediastinal disorders	Pulmonary hemorrhage, hemoptysis
Gastrointestinal disorders	Colitis, hematemesis, gastrointestinal pain
Skin and subcutaneous disorders	Petechie

(Panobinostat Investigator Brochure 2020[60], Farydak® (panobinostat) Package Insert[70])

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 recording AEs in the RAVE database. The descriptions and grading scales found in the revised NCI Common Terminology Criteria for (CTCAE) version 5.0 will be utilized for expedited AE reporting beginning April 1, 2018. All appropriate treatment areas should have access to a copy of the CTCAE versions 4.0 and 5.0. A copy of the CTCAE versions 4.0 and 5.0 can be downloaded from the CTEP web site http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

- **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*

Expedited AE reporting for this study must use CTEP-AERS (CTEP Adverse Event Reporting System), accessed via the CTEP Web site (https://eapps-ctep.nci.nih.gov/ctepaers). 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 **Table 15** and
Table 16.

In the rare occurrence when Internet connectivity is lost, a 24-hour notification is to be made by telephone at 901-595-5762 to the PBTC Operations Office. 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.

7.3.1 Distribution of Adverse Event Reports

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. For this protocol the following groups must be copied on expedited reports (24-hour notification and the complete report) submitted via CTEP-AERS:

IND Holder:
Center for Cancer Research
National Cancer Institute
[redacted]

Study Chair:
Michelle Monje, MD PhD
[redacted]

PBTC OBDMC:

Suresh (Rama) Ramanathan
Protocol Coordinator- Pediatric Brain Tumor Consortium
[redacted]

Study Co-Chair:
Kathy Warren, M.D.
[redacted]

John Glod, M.D.
[redacted]

The PBTC Operations Office will send the SAE/ CTEP-AERS report, within 24 hours of their awareness of the event to Secura Bio Inc. by email [redacted].

The IND Holder, NCI Center for Cancer Research, shall notify the FDA of any event that is both a serious suspected adverse reaction and unexpected in accordance with FDA rules and regulations (21 CFR 312.32). Follow-up information to a safety report will be submitted, as requested.

The IND Sponsor or designee will submit a report of the unexpected, suspected adverse event to the FDA using the FDA's reporting form (FDA 3500A MedWatch Form) and guidelines. The report should describe the event as fully as possible. Supporting documentation (lab reports, summary notes, and autopsy report) should accompany the report. A fatal or immediately life-threatening suspected adverse event will be reported to the FDA within 7 calendar days of the receipt of the initial report by the IND sponsor. A non-fatal, non-life threatening unexpected, suspected, serious adverse event will be reported to the FDA within 15 calendar days of receipt of the initial report by the IND Sponsor.

The PBTC OBDMC will post all IND Safety Letters on the PBTC-047 webpage. Sites will be notified via email of the receipt of the IND Safety Letter(s) and instructed to submit these to their local IRB in accordance with the institution‘s requirements.

A copy of the Annual Progress Reports is submitted by the IND holder as required by FDA, European Union (EU), Pharmaceutical and Medical Devices agency (PMDA) or other local regulators by the sponsor-investigator. Additionally, a copy of these reports will be submitted to CTEP at the time of submission to the appropriate regulatory agency.

7.3.2 Expedited Reporting Guidelines

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

Note: A death on treatment 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 "Disease progression"** in the system organ class (SOC) "General disorders and administration site conditions." 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]

Table 15

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

7.3.3 Additional Protocol-Specific Expedited Adverse Event Reporting Exclusions

For this protocol only, the AEs/grades listed below do not require expedited reporting via CTEP-AERS. However, they still must be reported through the routine reporting mechanism (Section 7):

Table 16

CTCAE SOC	Adverse Event	Grade	Hospitalization/ Prolongation of Hospitalization	Attribution	Comments
Investigations	Lymphopenia	≤ 4	Not required	Any	

7.3.4 Routine Adverse Event Reporting

All Adverse Events **must** be reported in routine (CTMS or CDUS) study data submissions. AEs reported through CTEP-AERS must also be reported in routine study data submissions.
Adverse event data collection and reporting, which are required as part of every clinical trial, are

done to ensure the safety of patients enrolled in the studies as well as those who will enroll in future studies using similar agents. AEs are reported in a routine manner at scheduled times during the trial using Medidata Rave. For this trial the Adverse Event CRF is used for routine AE reporting in Rave.

7.3.5 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 such as acute myelocytic leukemia (AML)
- 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.

7.3.6 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.

7.3.7 NCI Guidance for Reporting Protocol Deviations

The site must enter all protocol deviations into the PBTC RAVE database. Participating centers must also submit the report to their IRB in accordance with their institutional policies. Protocol deviation data entered in the PBTC RAVE database will be imported periodically to the NCI Protocol Support Office.

8 Agent Information

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

8.1 *Panobinostat*

Chemical Name: 2-Hydroxypropanoic acid, compd. with 2(E)-N-hydroxy-3-[4-[[[2-(2-methyl-1H-indol-3-yl)ethyl]amino]methyl]phenyl]-2-propenamide (1:1)

Other Names: (LBH589-lactate); Farydak®

Classification: Histone Deacetylase (HDAC) Inhibitor

CAS Registry Number: 404950-80-7

Molecular Formula: C21H23N3O2·H2O monohydrate

M.W.: 439.50416 g/mol

Mechanism of Action: May include effects on epigenetic mechanisms of gene expression as well as modulation of acetylation and function of histones

Formulation: Oral formulation: hard gelatin capsules contain panobinostat lactate salt at dosage strengths of 5 mg, 10 mg, 15 mg and 20 mg as anhydrous free base. The 5mg and 15mg capsules are packaged in high-density polyethylene (HDPE) bottles with an induction seal and plastic CR closure. The 10mg and 20mg formulations are packaged in blister packs with 6-ct capsules/pack. The 5 mg formulation will no longer be available after July 2021.

Storage: Store in a very tight packaging with water permeation <0.5 mg/day/liter, protect from light. Store below 25°C.

Stability: The shelf life for the capsule formulations is assigned based on ongoing stability studies.

Availability: Panobinostat capsules are provided by Secura Bio Inc.

Route of Administration: Capsules are to be taken orally by mouth and swallowed whole.

8.2 *Agent Ordering*

Panobinostat may be requested by the Principal Investigator or designees at each participating institution. All regulatory documents, as required by the PBTC, must be current and up to date prior to requesting the study drugs. Drug request form can be found on the PBTC-047 protocol and resources webpage under https://www.pbtc.org/members/Protocols/protocols.htm. The form can be emailed to [redacted] and [redacted] at [redacted] at Secura Bio Inc. (Phone number [redacted]).

The investigator, or a responsible party designated by the investigator, must maintain a careful record of the inventory and disposition of all agents, using the NCI Drug Accountability Record Form (DARF). See the CTEP home page at http://ctep.cancer.gov or the NCI Investigator's Handbook for Procedures for Drug Accountability and Storage.

8.3 *Agent Inventory Records*

Study personnel must ensure that all study medication and supplies are kept in a secure locked area with access limited to authorized personnel. This study product must not be used outside the context of this protocol. Under no circumstances should the investigator or site personnel supply study product to other investigators or clinics or allow the supplies to be used other than as directed by this protocol.

The investigator and/or the responsible site personnel must maintain accurate records of the receipt of all study medication shipped by National Cancer Institute or its designee, including, but not limited to, the date received, lot number, amount received, and the disposition of all study medication. Sites may use the NCI Drug Accountability Record Form (DARF) for this purpose. Current dispensing records must also be maintained that include the date and amount of medication dispensed, relevant batch or bottle code numbers, and study number assigned to the subject. The NCI DARF can be found at (http://ctep.cancer.gov/protocolDevelopment/requisition_agents/docs/accountability.pdf), the NCI DARF can also be found on the PBTC website under Generic Forms and Templates.

9 Pathology, Biomarker, Correlative, and Neuro-Imaging

This section contains the collection, shipping and handling information for all planned biomarker and exploratory correlative studies, neuropathology review and research imaging. The table below identifies the tests, sample type and amount, analyzing laboratory and whether it is required or optional. For additional details, please review the associated section below.

Table 17

Test Name	Sample Type	Analyzing Laboratory	Required or Optional	Section ID
PBTC Repository	FFPE tumor material and blood	Children's Hospital Los Angeles	Optional	**9.1**
Pharmacokinetics	Plasma	Figg Lab, NCI	Required	**9.2**
Pharmacodynamics: Cell-free DNA	Blood	McGill University	Optional	**9.3**

9.1 ***Pathology Central Review and Biorepository***

The Pathology Central Review and Biorepository (CRB)'s function is to collect, distribute and store specimens for central pathology review and planned correlative studies which support the laboratory objectives of this protocol.

The CRB will also serve as a central repository for specimens collected for future research and left over specimens (tumor tissue, blood, urine or CSF) returned to the repository following the planned analysis from patients who consent to long term storage of unused specimen. These samples will be stored in the repository for undefined future studies which support the mission of the PBTC. **If the patient does not consent to participation in the repository, correlative study samples should be submitted following the guidelines in the appropriate correlative study section below. If the patient does not consent to long term storage, remaining correlative study samples will be destroyed once the PBTC-047 analysis is complete.**

9.1.1 PBTC CRB Submission Guidelines

If the patient consents to provide slides for submission to the repository at the time of participation in a PBTC trial the following should be submitted:

- Tumor material
  Slides from the original and/or recurrent surgery should be prepared for storage. The site should provide up to twenty (20) unstained sections cut at 4µm in thickness on (+) slides from the most representative section. Fewer unstained sections may be submitted based on size and availability of tissue. Preference is for tissue that has not previously been frozen. The corresponding pathology report(s) including immunohistochemical, special stains, and molecular/genetic results is to be uploaded to the PBTC using the secure File Upload system. These reports will be made available to the pathologist via a link in the ProtoLab.

Suitability of sections would be established by preparing one (1) H&E to ensure that the sections meet the following criteria:

- Histologically representative of the reported lesion
- Contain at least 60% viable tumor
- No more than 40% necrosis

- Pre-Treatment Peripheral Blood Mononuclear Cells and Plasma
  PBMC may be collected by processing a 2-5ml whole blood specimen with Ficoll or collecting the specimen in a BD Vacutainer™ CPT™ Cell Preparation Tube with Sodium Citrate as noted below. Once separated, all pellets must be snap frozen and stored at least at -20ºC prior to dry ice shipment

  Specimen Collection and Processing by Ficoll tube
  - Collect 2 - 5ml of fresh blood into an EDTA tube.
  - Transfer blood into a sterile 50-mL tube and add double the amount of PBS. MIX GENTLY.
  - Set up another tube containing half of its total volume of Ficoll. For example, if there is 15 mL of blood + PBS, then use 7.5 mL of Ficoll (2:1 ratio).
  - At very slow pace (approx. 2 mL/minute), layer the blood + PBS mixture onto the Ficoll so that the solutions DO NOT MIX. Spin the blood/Ficoll at 750 g in slow mode for 30 minutes @ 25ºC. After spin you will see four distinct layers: plasma (top layer), white fluffy ring (2nd layer), Ficoll (3rd layer), and blood (bottom layer).
  - Remove plasma layer down to about 1 mL above the white fluffy ring and dispense into cryovials. Freeze the cryovials within 1 hour of collection and store immediately at -20°C or colder.
  - Collect the entire white fluffy ring. If ring is hard to see, also take extra liquid above. Then discard everything else.
  - Place this fraction of white blood cells into a fresh 50 mL sterile tube with 20 mL of PBS. Spin down for 10 minutes @ 25ºC, 750 g in fast mode. Remove the supernatant. Add back to pellet 1 mL of PBS and spin for 5 min. at 4°C at 10000rpm. Remove supernatant.
  - Freeze the pellet of WBCs in a 2ml cryovial and store at -80ºC until shipment.
  - Ensure that all tubes are clearly labeled with the PBTC patient accession number. Please ensure that the labeling system used is designed to withstand temperatures down to -80ºC. Samples should be stored at -80ºC until shipment. For short term storage (2-3 weeks) -20ºC is acceptable. NOTE 4ºC IS NOT ACCEPTABLE STORAGE.

  * If it is not possible to collect the PBMC by Ficoll gradient then separation of PBMC can be conducted using CPT tube separation as an alternative. However, the PBMC pellet MUST BE frozen immediately and stored at -80ºC.

  Collection and Processing by CPT tube
  - Peripheral blood should be collected in a BD Vacutainer CPT™ Cell Preparation Tube with Sodium Citrate. 8ml and 4ml CPT tubes can be obtained from Fisher

Scientific (Cat# 02-685-125, 02-688-81) or Becton-Dickinson (BD No.362761, 362760). The 8ml tubes have a 6ml minimum draw and the 4ml tubes have a 3ml minimum draw.
- Centrifuge the CPT™ tube at 1500 x g for 30 minutes at room temperature (20º C to 25º C). DO NOT APPLY THE BREAK ON THE CENTRIFUGE. Use acceleration 5, brake 0 ("slow mode").
- It may be necessary to spin the tube longer to ensure that all of the red blood cell components have been separated from the plasma layer through the polyester gel barrier.
- The tube should be removed immediately from the centrifuge. The mononuclear layer and plasma lie above the polyester gel plug.
- Using a sterile pipette, remove as much of the plasma component (upper half of the CPT tube) without disturbing the mononuclear layer if possible and aliquot it into cryovials. Freeze the cryovials within 1 hour of collection and store immediately at -20°C or colder.
- Transfer mononuclear cell layer (and some residual plasma layer) to a labeled 15-mL conical centrifuge tube and add 5ml sterile room temperature magnesium or calcium-free phosphate buffered saline (PBS) to fill the conical tube and recap.
- Centrifuge at 450 x g for 10 minutes at room temperature (20º C to 25º C). Use acceleration 9, brake ("fast mode").
- Remove supernatant, being careful not to aspirate the cellular pellet at the bottom of the tube.
- Add 1mL of sterile PBS to the pellet and gently re-suspend by pipetting up and down. Transfer the entire suspended pellet to the labeled 2ml cryovial.
- Centrifuge the cryovial at 450 x g for 5 minutes (or spin down the microcentrifuge tube at 1300 x g for 5 minutes) at room temperature. Discard the supernatant.
- Store the cell pellet cryovial frozen at -80ºC. For short term storage (2-3 weeks) -20º C is acceptable.

- If the patient consents to other secondary correlative studies as outlined in Section 9.3, the following specimens may also be submitted to the CRB for distribution and storage:
  - Peripheral blood cells: quantity and schedule of collection as outlined in Section 9.3.1 and 9.3.2

9.1.1.1 *Handling of Specimens*

- Slides are to be labeled with the study ID and the patient PBTC Accession # and these slides should be designated as PBTCR # (where the # assigned from 1 to 20, or the highest number of unstained sections prepared, sequentially) or PBTCR H&E for the H&E stained section.
- Scrolls or Formalin Fixed, Paraffin Embedded (FFPE) tumor materials are to be labeled with the PBTC Accession # and the PBTC study for which the sample is provided. Scrolls or FFPE tumor material should be shipped in a separate box (NOT with dry ice) at room temperature.

- Blood samples are to be labeled with the PBTC Accession # and the PBTC study for which the sample is provided. Samples should be shipped in a separate box with 2-day supply of dry ice per Section 9.3.2.
- Urine samples are to be labeled with the PBTC Accession # and the PBTC study for which the sample is provided. Samples should be shipped overnight on dry ice. Urine sample no longer collected as of version 8.0.

9.1.1.2 *Shipment of Specimens*

Samples collected for the repository should be sent to the PBTC CRB via FedEx by completing the Internet form at http://www.fedex.com/us/ and requesting FedEx to use the cc function to e-mail [redacted]. FedEx user ID and password for pathology shipping can be found at PBTC-047 protocol webpage. All specimens should be shipped to the below address, with a completed transmittal form which can be found on PBTC-047 webpage.

PBTC CRB
[redacted]

9.1.2 Pathology Central Review (Optional)

Central pathology review is *optional* for this study. In consenting patients, samples for central pathology review will be completed from the slides submitted to the PBTC CRB. In patients who have not consented to the PBTC CRB, slides may be submitted only for purposes of pathology central review for this protocol.

Pathologist review for this study will include the following elements:

- Examination of H&E stained slides and, if the tumor being reviewed is residual, recurrent or metastatic, examination of H&E slides from the original primary tumor. For each subject one H&E stained slide per one representative block from the brain tumor, removed either at initial diagnosis or relapse, should be submitted for review.
- Review of the corresponding pathology report(s) of the immunohistochemical, special stains, and molecular/genetic results from current and/or original primary tumor
- If necessary, review of immunohistochemical or special stained slides. Slides submitted to the PBTC CRB will be digitized to 40X. H&E stained sections will be retained and filed at the CRB. Original immunohistochemical or special stain slides will be returned to the submitting institution.

9.2 ***Pharmacokinetics – Plasma (Required)***

Plasma pharmacokinetic studies are mandatory and will be obtained from all patients enrolled on this study.

9.2.1 Collection of Specimens

Sampling Strategy for Pharmacokinetic Study (Note: Patients should take Panobinostat in a fasting state on Days 1 and 3 of Course 1 only)

- On Day 1 of Course 1, serial blood samples of 2 ml each for panobinostat pharmacokinetic studies will be collected at the following times: pre-dose and at 0.5, 1, 2, 4, 8 (±1), and 24 (±4) hours after the oral dose.
- On Day 3 of Course 1 (pre-dose 2), a single sample of 2 ml will be collected prior to the drug dose.

9.2.2 *Handling of Specimens*

At each time point, 2 ml of blood will be collected into appropriately labeled tubes containing Sodium Heparin. 2 ml of blood is needed to provide approximately 2 aliquots plasma for pharmacokinetic analysis. The Pharmacokinetic data collection form should be completed with the exact time that the sample is drawn as well as the exact time that the drug is administered. The PK data collection form is located on the PBTC-047 webpage.

During the sample collection and processing steps, the blood and plasma should be kept out of direct sunlight and unfiltered lab light. Upon collection of the blood PK samples in Na Heparin blood collection tubes, keep the blood samples on wet ice at all times prior to processing to plasma. Within 30 minutes of collection, samples will be centrifuged at approximately 2000 x g for about 10 minutes at 4°C (Note: If a refrigerated centrifuge is not available, please refer to the Shipping Manual located on the PBTC-047 webpage for further instructions). The plasma will be stored in an appropriately labeled amber screw-capped polypropylene tube and stored immediately at approximately -20°C.

9.2.3 Shipping of Specimens

Samples should be shipped for delivery Monday through Thursday with a generous amount of dry ice enclosed to safeguard against shipping delays. Weekend and holiday deliveries should be avoided. At the start of the study, each participating institution should refer to the PBTC-047 website for instructions to request a Pharmacokinetics Kit for each patient enrolled on the study.

Samples should be batched and shipped within 30 days of the last sample being taken. The site should use the PBTC-047 FedEx account details, located on the PBTC member website. Ship all pharmacokinetic samples frozen and on dry ice, along with a completed Pharmacokinetics Sample Transmittal Form located on the PBTC-047 webpage to:

Figg Lab
[redacted]

Samples must be shipped from the site to the respective receiving laboratory within 30 days of last sample collection in order to receive cost reimbursement. The sample collection and shipping dates must be documented in the eCRF. Samples will be stored at this receiving laboratory, until additional details regarding the analyzing laboratory are available, which will be included in a forthcoming amendment.

9.2.4 Assay description including Site Performing Correlative Study

The purpose of this study is to evaluate and characterize the plasma pharmacokinetics of panobinostat in children with recurrent/progressive and non-progressed DIPG or H3K27M+ Thalamic DMG. 100 μL of clinical sample in human plasma in Na heparin (or K_3EDTA) stored at -70ºC±10 is used in protein precipitation treatment (PPT). After sample treatment, the sealed plate is transferred onto auto-sampler of HPLC and then analyzed by an LC-MS/MS system. The dilution factors include 1/5, 1/20 and 1/50.

9.3 ***Pharmacodynamics: Cell-free DNA – Blood and Urine (Optional)***

As an ancillary/exploratory study in consenting patients, blood and/ or urine will be collected to detect histone mutation status in cell-free DNA, via Microfluidic analysis of cell-free DNA.

9.3.1 Collection of Specimens

- Blood Sample
  2-3 ml of serum peripheral blood for plasma will be collected on days 1 of courses 1, 2, 4, 6, and 12, pre-dose into K2-EDTA lavender top tubes.

- Urine Sample (No longer need to be collected as of version 8.0)
  3-5 ml of urine will be collected on days 1 of courses 1, 2, 4, 6, and 12 pre-dose, preferably in the morning time.

9.3.2 Handling and Shipping of Specimens

- Blood Sample
  At each time point, 2-3 ml of blood will be collected into appropriately labeled tubes containing K2-EDTA. Blood samples must be labeled with the PBTC accession number, protocol ID and date of collection. Samples should be batched for shipment once each patient's complete set has been collected and processed and shipped within 30 days of the last sample being taken. Blood samples should be shipped Monday through Wednesday via Federal Express Priority Overnight using the FedEx account information and transmittal form, located on the PBTC-047 webpage. Weekend deliveries are not permitted. Receipt and processing of biological materials will be recorded in ProtoLab, as

appropriate, by staff at the analyzing laboratory. The sites sending the materials must document sample collection and shipping dates in the eCRF.

During the sample collection and processing steps, the blood and plasma should be kept out of direct sunlight and unfiltered lab light. Upon collection of the blood cell-free DNA in K2-EDTA blood collection tubes, samples should be processed as soon as possible to minimize hemolysis and contamination due to DNA released from nucleated blood cells. Samples should be centrifuged at 800 x g for about 10 minutes at 4° C. The supernatants should then be transferred to new 1.5ml tubes, keeping the pellet for blood DNA extraction if needed. Then, the supernatants should be centrifuged at 16000 x g for about 10 minutes at 4° C. Aliquot the supernatants by 1ml and store at -80° C until use. Blood samples will be shipped overnight via Federal Express Monday through Wednesday with a 2-day supply of dry ice. Specimens will only be received Tuesday through Thursday.

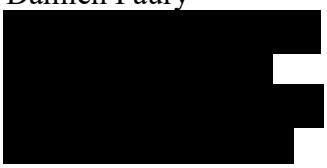
Specimens should be sent to the following address:

Damien Faury


Please notify Damien Faury of a pending shipment along with the FedEx tracking number by telephone.

- Urine Sample (No longer collected as of version 8.0)
  Collect 3-5 ml per time point, preferably in the morning to increase cfDNA concentration. Immediately freshly collected urine samples should be mixed with 0.5M EDTA pH 8.0 to a final concentration of 10mM EDTA (dilution 1/50), to inhibit possible nuclease found in urine. The samples should then be kept at -80°C until shipment. Urine samples must be labeled with the PBTC accession number, protocol ID and date of collection. Samples should be batched for shipment once each patient's complete set has been collected and processed and shipped within 30 days of the last sample being taken. Urine samples should be shipped Priority Overnight on dry ice via Federal Express Monday through Wednesday. Specimens will only be received Tuesday through Thursday.

  Specimens should be sent to the following address:

  Damien Faury
  

Please notify Damien Faury of a pending shipment along with the FedEx tracking number by telephone.

9.3.3 Assay description including Site Performing Correlative Study

The purpose of this study is to confirm on-target effects. Samples will be thawed on ice then up to 3 ml of plasma or 5 ml of urine will be processed. Cell-free nucleic acids will be extracted using the QIAmp circulating Nucleic Acid kit (Qiagen). Samples will be eluted in 20-30 ul of elution buffer and then quantified with Picogreen assay. A ddPCR (droplet digital PCR) duplex assay will be run on 5 ul of each extraction using the QX-100 system from BioRad. The conditions of the different assays have already been optimized to detect both wild-type (HEX probe) or mutant (FAM probe). Results will be analyzed using QuantaSoft software.

9.4 ***Neuroimaging Studies***

Patients will have MRI Brain with and without contrast with diffusion performed prior to therapy, every 2 courses for the first 6 courses, and after every 3 courses thereafter, until time of progression or completion of treatment. MRI Spine should be performed prior to therapy and at the same time points as standard MRI Brain, if clinically indicated. The MR parameters will be listed on the PBTC NIC web page under MR protocols. Only scans showing a response, the confirmation scan obtained approximately 8 weeks later if available, and the corresponding baseline scan will be uploaded for central review.

9.4.1 Imaging Guidelines

9.4.1.1 *Brain MR with and without gadolinium will be obtained preferably on the 3T magnet consisting of:*

- Sagittal T1 MPRAGE (slice thickness 1.0, 25 cm FOV)
- Axial T2 images (slice thickness 2mm skip 0, 20 cm FOV)
- Axial T2 FLAIR images (slice thickness 4mm skip 0, 20 cm FOV)
- Axial DTI images (slice thickness 2.0 mm skip 0, 22 cm FOV); 35 directions, b-values: 0 and 1000 s/mm^2
- Post gadolinium sagittal T1 SPACE (slice thickness 0.9 mm skip 0, 22 cm FOV)
- Axial T1 post gadolinium images through the whole brain (slice thickness 4mm skip 0, 20 cm FOV)

9.4.1.2 *Brain MR with and without gadolinium on 1.5 T:*

- Sagittal T1 (slice thickness 5 mm skip 1 mm, 22 cm FOV)
- Axial T2 images (slice thickness 4 mm skip 0 mm, 20 cm FOV)

- Axial T2 FLAIR images (slice thickness 5 mm skip 0 mm, 20 cm FOV)
- Axial DWI, 6 directions (slice thickness 5 mm skip 0 mm, 26 cm FOV)
- Post gadolinium sagittal 3DFSPGR images (slice thickness 1.5 mm no skip, 24 cm FOV)
- Axial T1 post gadolinium (slice thickness 3 mm no skip, 16 cm

9.4.1.3 *Protocol for Spine*

- Sagittal T1 images should be after gadolinium (slice thickness 3 mm skip 0).
- Axial T1 images are after gadolinium (slice thickness 3mm skip 0). Axial T2 images are optional

9.4.2 Image Transfer

Standard MRI with gadolinium and diffusion images obtained at each time point, including progression during the extended follow-up period if applicable, will be electronically transferred to the PBTC Neuroimaging Center (NIC). All patient specific data are stripped from the images and replaced with PBTC Accession numbers prior to transmitting the 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

9.4.3 Neuroimaging Review

Local review of MR imaging studies at each site and central review of the MR imaging studies will be conducted through the PBTC Neuroimaging Center (NIC). The NIC will review the imaging studies at study completion.

NIC review will include assessment of response to therapy (as feasible). All patients with a documented PR/CR at any time point will have central review of the scan showing response, the confirmation scan obtained approximately 8 weeks later, as well as the corresponding baseline scan conducted for confirmation.

9.5 ***Autopsy Studies***

Sites are encouraged to obtain an autopsy when possible and appropriate on enrolled patients. Performing pathologists will be requested to look for evidence and extent of immune cell infiltration in the specimen.

Specifically, it is recommended that the following assessments be made and documented in the autopsy report:

9.5.1 Determine areas of necrosis and attempt analysis for cause/etiology, if possible.

9.5.2 Determine the extent of lymphocyte infiltration particularly in adjacent areas of edema / inflammation.

In addition, 15-20 slides will be requested to perform staining for characterization of infiltrating lymphocyte population and other studies. Please ensure this is covered in your site's autopsy consent form.

10 Study Calendar

Data is to be submitted according to the Data submission timelines located on the PBTC-047 webpage.

Table 18

	Pre-therapy	Course 1	Courses 2-Course 26	Completion/ Discontinuation Of Treatment	Off Tx Follow-up
Physical Assessments					
Panobinostat Administration, patients with recurrent/progressive DIPG (Stratum 1)		1 x/day, 3x/week every other day for 3 weeks. 1 week rest	1 x/day, 3x/week every other day for 3 weeks. 1 week rest		
Panobinostat Administration, patients with non-progressed DIPG or H3K27M+ Thalamic DMG (Stratum 2)		1 x/day, 3x/week every other week	1 x/day, 3x/week every other week		
Medical history	X	Weekly	X	X	
Physical exam /height/weight	X	Weekly	X	X	
Vital signs	X	Weekly	X	X	
Performance status	X		X	X	
Neurologic exam	X		X	X	
Laboratory Evaluations					
CBC WBC, HgB, Platelets, ANC, ALC [A]	X	Weekly	X	X	
Serum Chemistry Sodium, Potassium, Bicarbonate, Chloride, Calcium, BUN, Creatinine, Glucose, Phosphorous, Magnesium, Albumin, Total Protein, SGPT(ALT), SGOT(AST), Total Bilirubin	X	X	X	X	
Serum or Urine pregnancy test (for females of childbearing potential)	X	X	X		
TSH, free T_4	X			X	
Other Assessments					
EKG [A]	X	Day 1, and Day 5 (3^{rd} dose): Pre-dose and 3 hrs post dose	Pre-dose Day 1 of Course 2,3,4		
Echocardiogram or MUGA	X			X	
Imaging Assessments					

	Pre-therapy	Course 1	Courses 2-Course 26	Completion/ Discontinuation Of Treatment	Off Tx Follow-up
Brain MRI (standard) [B]	X		After every 2 courses for the first 6 courses, then after every 3 courses until progression	End of Tx or progression	Every 3 months until progression
Brain MRI with diffusion [B]	X		After every 2 courses for the first 6 courses, then after every 3 courses until progression	End of Tx or progression	Every 3 months until progression
Spinal MRI (if clinically indicated) [B,C]	X		After every 2 courses for the first 6 courses, then after every 3 courses until progression	End of Tx or progression	Every 3 months until progression

[A] This should also be done whenever clinically indicated.
[B] All required imaging dates are to be entered in the database. However, only scans showing a partial response or complete response, and that same subject's baseline scan, will be uploaded.
[C] MRI Spine should be done at the indicated time and when clinically indicated. If no disease is present within the spine at baseline, spine MRIs will be obtained as clinically indicated and at the discretion of the primary treating physician.

Table 19

Correlative Laboratory Studies	Pre-therapy	Course 1	Course 2, 4, 6, 12
Tumor Tissue and Blood for Biorepository (optional)	**X**		
Pharmacokinetics **Plasma** (required)		• Day 1: pre-dose and at 0.5, 1, 2, 4, 8 (±1), and 24 (±4) hours after the oral dose. • Day 3 (2nd dose): single sample will be collected prior to the dose	
Pharmacodynamics: Cell-free DNA – **Blood** (optional)		Day 1, Courses 1, 2, 4, 6, 12, prior to drug dose (Optional Biorepository: Leftover Sample Storage for Future Research)	
Pharmacodynamics: Cell-free DNA – **Urine** (optional)		Day 1, Courses 1, 2, 4, 6, 12, prior to drug dose, preferably in morning (No longer collected as of version 8.0)	

11 Measurement of Effect

Although the clinical benefit of panobinostat has not yet been established, the intent of offering this treatment is to provide a possible therapeutic benefit, and thus the patient will be carefully monitored for tumor response and symptom relief in addition to safety and tolerability. Patients with measurable disease will be assessed by standard criteria. For the purposes of this study, patients should be re-evaluated by MRI after every 2 courses for the first 6 courses, then every 3 courses and when clinically indicated until disease progression or off-study criteria are met.

11.1 *Definitions*

11.1.1 Evaluable for Toxicity

Patients who receive at least 1 dose of the study regimen and are removed from treatment for toxicity during the first course (dose-finding period) are evaluable for estimating the MTD.

Patients who receive approximately 83-85% of prescribed therapy (i.e. ≥8/9 doses in stratum 1 and ≥5/6 doses in stratum 2) during the dose-finding period but who progress prior to completing the course 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 must 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 estimation.

Patients who receive less than 83-85% of the protocol specified therapy (i.e. miss >1 dose) and who go off treatment for reasons other than toxicity (e.g., progressive disease, withdrawal of consent etc.) during the dose finding period will be considered inevaluable for estimating the MTD and may 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 first course may be considered inevaluable for estimating the MTD and may be replaced.

11.1.2 Evaluable for Radiographic Response

Only those patients who have measurable disease present at baseline, have received at least one course of therapy, and have had their disease re-evaluated will be considered evaluable for response. These patients will have their response classified according to the definitions stated below. (Note: Patients who exhibit objective disease progression prior to the first scheduled MRI will also be considered evaluable.)

11.2 *Tumor Response Criteria*

11.2.1 Complete Response (CR)

Complete disappearance on MR of all evaluable 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. If CSF was positive, it must be negative.

11.2.2 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. *Axial FLAIR images will be used for tumor measurements.*

11.2.3 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

11.2.4 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 wean, 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 tumor lesion.

Increasing doses of corticosteroids required to maintain stable neurological status should be strongly considered as a sign of clinical progression unless in the context of recent wean or transient neurologic change due e.g. to radiation effects.

11.2.5 Progression-Free Survival (PFS)

Interval of time between date of initiation of protocol treatment and minimum date of documentation of PD, death due to any cause, or date of last follow-up.

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*

Medidata Rave is a clinical data management system being used for data collection for this trial. Access to the trial in Rave is controlled through the CTEP-IAM system and role assignments.

Requirements to access Rave via iMedidata:

- A valid CTEP-IAM account; and
- Assigned a Rave role on the PBTC roster at the enrolling site of: Rave CRA, Rave Read Only, Rave CRA (LabAdmin), Rave SLA, or Rave Investigator.

Rave role requirements:

- Rave CRA or Rave CRA (Lab Admin) role must have a minimum of an Associate Plus (AP) registration type;
- Rave Investigator role must be registered as an Non-Physician Investigator (NPIVR) or Investigator (IVR); and
- Rave Read Only role must have at a minimum an Associates (A) registration type.

Refer to https://ctep.cancer.gov/investigatorResources/default.htm for registration types and documentation required.

Upon initial site registration approval for the study in Regulatory Support System (RSS), all persons with Rave roles assigned on the appropriate roster will be sent a study invitation e-mail from iMedidata. To accept the invitation, site staff must log in to the Select Login (https://login.imedidata.com/selectlogin) using their CTEP-IAM username and password and click on the *accept* link in the upper right-corner of the iMedidata page. Site staff will not be able to access the study in Rave until all required Medidata and study specific trainings are completed. Trainings will be in the form of electronic learnings (eLearnings) and can be accessed by clicking on the link in the upper right pane of the iMedidata screen. If an eLearning is required and has not yet been taken, the link to the eLearning will appear under the study name in iMedidata instead of the *Rave EDC* link; once the successful completion of the eLearning has been recorded, access to the study in Rave will be granted, and a *Rave EDC* link will display under the study name.

Site staff that have not previously activated their iMedidata/Rave account at the time of initial site registration approval for the study in RSS will receive a separate invitation from iMedidata to activate their account. Account activation instructions are located on the CTSU website in the Data Management section under the Rave resource materials (Medidata Account Activation and Study Invitation Acceptance). Additional information on iMedidata/Rave is available on the CTSU members' website in the Data Management > Rave section at www.ctsu.org/RAVE. or by contacting the CTSU Help Desk at 1-888-823-5923 or by e-mail at ctsucontact@westat.com.

12.2 *Method*

This study will be monitored using CDUS-Abbreviated reporting, and as such no adverse event reporting (routine or expedited) is required to be reported via CDUS.

12.3 *Responsibility for Data Submission*

The OBDMC for the PBTC is responsible for compiling and submitting CDUS data to CTEP for all participants and for providing the data to the Principal Investigator and sponsor for review.

12.4 *CTEP Multicenter Guidelines*

The specific responsibilities of the Principal Investigator and the Coordinating Center:

- The Principal Investigator/Coordinating Center is responsible for distributing all IND Action Letters or Safety Reports received from the pharmaceutical company to all participating institutions for submission to their individual IRBs for action as required.

- Except in very unusual circumstances, each participating institution will order agents directly from the Secura Bio Inc. Agents may be ordered by a participating site only after the initial IRB approval for the site has been forwarded by the Coordinating Center to the sponsor.

12.5 *Collaborative Agreements Language*

A Clinical Trial Agreement will be in place to document study operations between Secura Bio Inc. and the PBTC. A Materials Transfer Agreement will be in place to document correlative study operations between the applicable labs and analyzing centers, and the PBTC.

12.6 *Genomic Data Sharing Plan*

Unlinked genomic data will be deposited in public genomic databases such as dbGaP in compliance with the NIH Genomic Data Sharing Policy.

13 Statistical Considerations

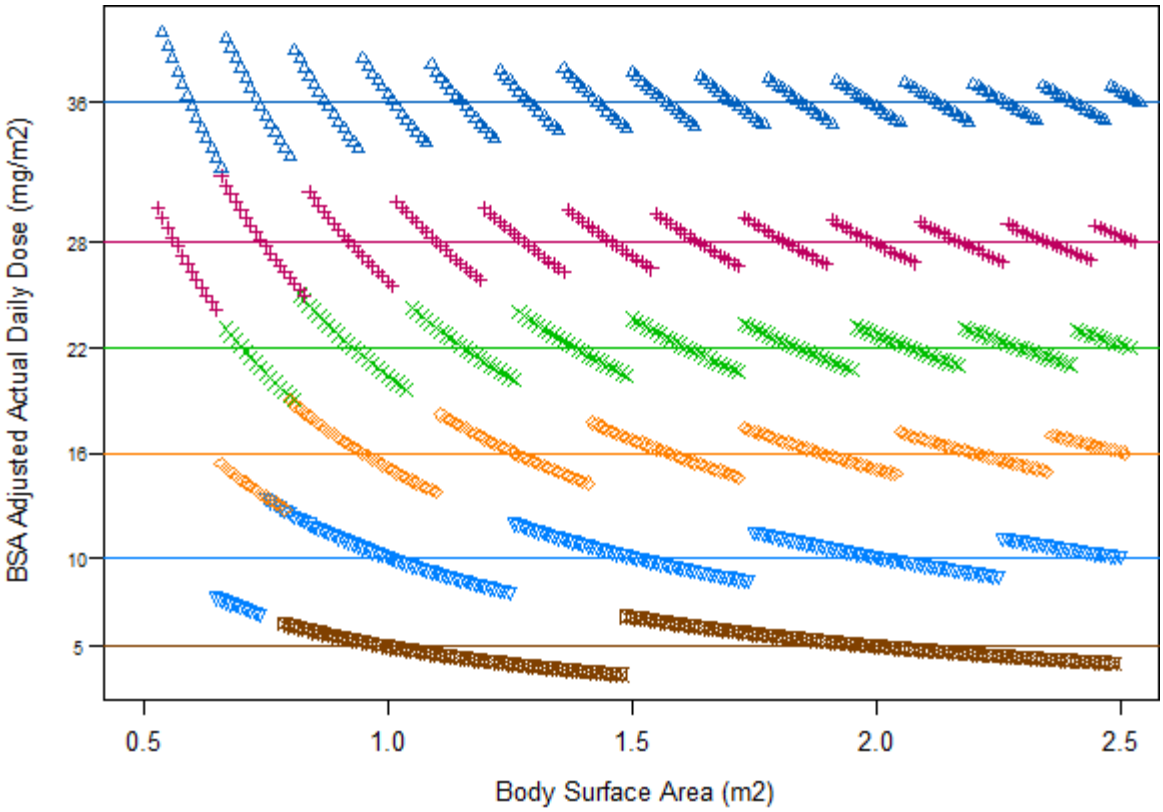
13.1 *Study Design/Endpoints*

Six dose levels are proposed and all may or may not be investigated, primarily dependent upon whether or not toxicity at dose level 1 results in de-escalation to dose level 0. Enrollment will commence at dose level 10mg/m^2 for Stratum 1 and at 1 dose level above the Stratum 1 MTD/RP2D in Stratum 2. If the '3 weeks on, 1 week off' schedule is abandoned in Stratum 1 without determining the MTD, then we will initiate dose escalation in Stratum 2 at 10mg/m^2. Dose finding will be governed by a two stage continual reassessment method (CRM) which will be applied separately in the two strata. No intra-patient dose escalations will be allowed.

13.1.1 Proposed Dose Levels and BSA Based Dosing

Since panobinostat is an oral agent and supplied in fixed capsule sizes, the deliverable doses based on BSA considerations are limited to the rounding constraints as set by the minimum capsule size. Taking this into consideration along with the starting dose of 10mg/m^2 in Stratum 1 we developed an algorithm that enabled us to choose dose levels that would avoid overlaps in BSA adjusted doses in our patient cohorts. The following plot illustrates the BSA adjusted doses that the patients would receive at the proposed dose levels based on a minimum capsule size of 5mg. For the lower dose levels we have also utilized minimum BSA restrictions for enrollment to avoid these overlaps. Dosing nomograms are provided in **Appendix D: Dosing Tables** to facilitate correct dosing of patients based on this approach.

Figure 2: BSA Adjusted Doses vs. Body Surface Are for the Proposed Dose Levels



13.1.2 Dose Finding Algorithm

Likelihood Based 2-Stage CRM:
In this trial we will use the likelihood-based 2-stage CRM[71,72,73] as the dose finding algorithm for estimating the MTD. We will use the *dfcrm* package[71] available in R to operationally execute this approach. The toxicity probability of 0.25 is determined as the target probability to be associated with the MTD for both strata. That is, the MTD is defined as the dose at which the model estimates that 25% of patients would be expected to experience dose-limiting toxicities (DLT).

With pre-determined dose levels to be investigated, the practical implementation is to call the "MTD" the proposed dose level nearest to the value identified by the model (dose-finding MTD). The trial will continue until the MTD recommendation can be made based on at least 6 patients treated at that dose level; the model recommends a level lower than the lowest proposed level; or 6 patients have been studied at the highest pre-determined dose level and the model suggests escalation. In the event that the model recommends a level lower than the lowest proposed level or 6 patients have been studied at the highest pre-determined dose level and the model suggests escalation, an estimate of the MTD will not be available, though a Phase II dose may still be recommended utilizing also the available PK and clinical data. If the trial results in an MTD, the reports of this study will include the model estimated MTD as well as the "dose-finding MTD" based on the fixed proposed levels.

We will initiate the dose finding algorithm, so called the *first-stage*, using an empirical approach until the first DLT is observed. Once the first DLT is observed, we will switch to a CRM design (*second stage*) and will use a likelihood-based algorithm to guide dose finding. Since we are using

a likelihood based approach, the use of the first stage is needed as the model cannot estimate the MTD unless some heterogeneity is observed in the toxicity outcomes i.e. at least 1 DLT and 1 non-DLT.

Details of the Dose Finding Algorithm for Stratum 1:

First Stage:
We will initiate enrollment at dose level 1 and in the absence of any DLTs we will enroll the following numbers of patients at each dose level. This enrollment sequence has been shown to be coherent[71] for the target probability of 25%. Note that enrolling 3 patients at dose level 1 would make this enrollment sequence non-coherent:

Planned Enrollment per Dose Level in the absence of any DLTs

Dose level	0	1	2	3	4	5
Number of Patients to be Enrolled	0	2	3	3	3	6

Until the first DLT is observed, using an approach similar to empirical methods e.g. 3+3 or Rolling-6, we will make the above-described number of slots available at each dose level and will wait until all patients have completed the DLT assessment before escalating to the next dose level. In the absence of a DLT in the prior dose levels, once we reach the highest dose, we will make 6 slots available.

Second Stage:
Once the first DLT is observed, we will switch to a CRM design (*second stage*) and will use a likelihood-based algorithm via the empiric function (also called the power function, $F(x,\beta)=x^{\beta}$ *for* $0<x<1$) to guide dose finding. This function has been shown to perform well under a wide variety of scenarios using the two-stage CRM. Following the detailed guidance in Cheung et al, 2011[71] and using 25% as target probability for the MTD, we have calibrated this design via the following statistical parameters:

- An indifference interval was defined with a half width of 7% i.e. if the model identifies a dose as the MTD in the interval 18% to 32% (25%±7%), the investigators will be indifferent to this choice.
- Dose level 3 is chosen as the initial guess for the MTD in line with the recommendations in Cheung et al, 2011[71].
- Based on the above assumptions, the skeleton (initial guesses for the toxicity probabilities) is estimated as the following:

The initial toxicity probability assumptions (the skeleton)

Dose level	0	1	2	3	4	5
Toxicity probability	0.02	0.05	0.13	0.25	0.40	0.54

- MTD will be declared as the dose level at which at least 6 patients have been treated and the algorithm does not recommend escalation or de-escalation.
    - Once the MTD is declared or the Phase II recommended dose is identified, we will enroll 6 additional patients at this dose level to better characterize the toxicity and pharmacokinetic profile of this dose level. We will monitor the DLTs in this

expanded cohort in a similar fashion and if the toxicities in this expanded cohort suggest that the initially declared MTD may be too toxic, we will reduce the dose level and study additional patients at the lower dose until 12 patients have been treated at this dose level and the model does not suggest de-escalation.

While the purest form of the CRM calls for fully sequential dose assignments, we will implement some restrictions in an effort to be conservative in dose escalations:

- We will escalate 1 dose level at a time without skipping doses.
- Once the second-stage is initiated, at each new dose level three slots will be made available and we will require that all patients complete the DLT assessment before additional patients can enroll. Furthermore, we will restrict enrollment so that no more than 3 patients will have DLT evaluation pending at any given point in time.
- Once a dose level has been visited once and 2 evaluable patients have been treated there, subsequent patients will be assigned to the dose level closest to the current estimate of the MTD without skipping a level, if the decision is to escalate. In the event that the model recommends de-escalation, skipping dose levels is permitted. If the first dose level (dose level 1) is found to have unacceptable toxicity, additional patients will be treated at dose level 0.
- It is typical to encounter study eligible patients who are initially enrolled to the dose-finding phase and are later deemed inevaluable for dose-finding for some reason, such as incomplete course of treatment. Our version of the CRM requires that at least two patients are not only enrolled at a given previously unstudied dose level but are also evaluable for dose-finding. If two patients are enrolled to a level and one is deemed inevaluable for dose-finding, then the inevaluable patient must be replaced to ensure that at least two evaluable patients have been treated at that level, regardless of the outcome of the evaluable patient, unless the algorithm recommends de-escalation following a DLT outcome from the first patient.
- Per CTEP's recommendation we will also employ the following 2 restrictions in an attempt to minimize exposure of patients to dose levels that may be too toxic:
    - New patients will not be assigned to a dose level **above** a dose level where ≥ 33% of patients have experienced a DLT.
    - New patients will not be assigned to a dose level **at which** 4 or more patients have been treated and ≥ 33% of the patients have experienced a DLT.

Details of the Dose Finding Algorithm for Stratum 2:

First Stage:
We will initiate enrollment 1 dose level above the highest safe dose studied in Stratum 1 (denoted generically as dose level 1 below) and in the absence of any DLTs we will enroll the following numbers of patients at each dose level.

Planned Enrollment per Dose Level in the absence of any DLTs

Dose level	1	2	3	4
Number of Patients to be Enrolled	3	3	3	6

Until the first DLT is observed, using an approach similar to empirical methods e.g. 3+3 or Rolling-6, we will make the above-described number of slots available at each dose level and will wait until all patients have completed the DLT assessment before escalating to the next dose level. In the absence of a DLT in the prior dose levels, once we reach the highest dose, we will make 6 slots available.

Second Stage:
Once the first DLT is observed, we will switch to a CRM design (*second stage*) and will use a likelihood-based algorithm via the empiric function (also called the power function, $F(x,\beta)=x^{\beta}$ *for* $0<x<1$) to guide dose finding. This function has been shown to perform well under a wide variety of scenarios using the two-stage CRM. Following the detailed guidance in Cheung et al, 2011[71] and using 25% as target probability for the MTD, we have calibrated this design via the following statistical parameters:

- An indifference interval was defined with a half width of 7% i.e. if the model identifies a dose as the MTD in the interval 18% to 32% (25%±7%), the investigators will be indifferent to this choice.
- Dose level 2 is chosen as the initial guess for the MTD in line with the observed toxicity information from Stratum 1
- Based on the above assumptions, the skeleton (initial guesses for the toxicity probabilities) is estimated as the following:

The initial toxicity probability assumptions (the skeleton)

Dose level	0	1	2	3	4
Toxicity probability	0.05	0.13	0.25	0.40	0.54

- MTD will be declared as the dose level at which at least 6 patients have been treated and the algorithm does not recommend escalation or de-escalation.
    - Once the MTD is declared or the Phase II recommended dose is identified, we will enroll 6 additional patients at this dose level to better characterize the toxicity and pharmacokinetic profile of this dose. We will monitor the DLTs in this expanded cohort in a similar fashion and if the toxicities in this expanded cohort suggest that the initially declared MTD may be too toxic, we will reduce the dose level and study additional patients at the lower dose until 12 patients have been treated at this dose level and the model does not suggest de-escalation. If the accrual is slow and the expansion cohort does not reach 12 subjects in a reasonable time and if the DLT outcome of the additional patients will not affect the MTD estimate, enrollment may be declared as complete. This decision will be made by the study team, IND Sponsor and the PBTC leadership in collaboration with the PBTC DSMB.

While the purest form of the CRM calls for fully sequential dose assignments, we will implement some restrictions in an effort to be conservative in dose escalations:

- We will escalate 1 dose level at a time without skipping doses.
- Once the second-stage is initiated, at each new dose level three slots will be made available and we will require that all patients complete the DLT assessment before additional patients can enroll unless the DLT information from the pending patients do not change the escalation/de-escalation decision.

- Once a dose level has been visited once and at least 2 evaluable patients have been treated there, subsequent patients will be assigned to the dose level closest to the current estimate of the MTD without skipping a level. If the first dose level (dose level 1) is found to have unacceptable toxicity, additional patients will be treated at dose level 0.
- It is typical to encounter study eligible patients who are initially enrolled to the dose-finding phase and are later deemed inevaluable for dose-finding for some reason, such as incomplete course of treatment. Our version of the CRM requires that at least two patients are not only enrolled at a given previously unstudied dose level, but are also evaluable for dose-finding. If two patients are enrolled to a level and one is deemed inevaluable for dose-finding, then the inevaluable patient must be replaced to ensure that at least two evaluable patients have been treated at that level, regardless of the outcome of the evaluable patient, unless the algorithm recommends de-escalation following a DLT outcome from the first patient.
- Per CTEP's recommendation we will also employ the following 2 restrictions in an attempt to minimize exposure of patients to dose levels that may be too toxic:
    - New patients will not be assigned to a dose level **above** a dose level where ≥ 33% of patients have experienced a DLT.
    - New patients will not be assigned to a dose level **at which** 4 or more patients have been treated and ≥ 33% of the patients have experienced a DLT.

13.1.3 Contingencies

If all proposed levels are investigated with acceptable toxicity, consideration will be given to amending the protocol to include higher dose levels. This deliberate decision will be made by the study committee and the sponsor and will be implemented via an amendment. In the event that higher doses are not studied and 12 patients have been treated at the highest dose level without the CRM suggesting de-escalation, then the highest dose level may be recommended for further study in subsequent trials.

In the event of a single fatal drug-related adverse event, the OBDMC will suspend registration pending discussion with the Study Chair, the PBTC DSMB, and others as necessary.

13.2 *Sample Size/Accrual Rate*

The projected accrual rate is 1-2 patients per month, based on prior PBTC trials for similar patient populations. With 2 dose levels studied in Stratum 1 and possible 4 dose levels (we believe it is unlikely we will study both dose level 0 and dose level 4) in Stratum 2, the total sample size is expected to be approximately 40-50 patients accrued in approximately 1.5-2 years. These estimates do not take into account any unusual circumstances such as initial MTD turning out to be too toxic following additional toxicities in the expansion cohort etc. The gender and minority distribution of the study population is expected to be as follows in **Table 20**:

Update for Version 8:
At the time of drafting of version 8 of the protocol, dose level 3 in Stratum 2 was declared as too toxic after the expansion cohort. When de-escalation to dose level 2 was initiated, 47 patients had been enrolled on the trial: 6 of those were treated at dose level 2 of Stratum 2 and 3 were treated

at dose level 1 of Stratum 2, Thus, in order to adequately assess the tolerability of dose level 2 or dose level 1 if further de-escalation is needed, with this amendment the maximum sample size in this trial will be increased to 70. This maximum sample size also accounts for potential inevaluable subjects.

Table 20: Gender and Minority Accrual Estimates

PLANNED ENROLLMENT REPORT

Racial Categories	**Ethnic Categories**				**Total**
	Not Hispanic or Latino		**Hispanic or Latino**		
	Female	**Male**	**Female**	**Male**	
American Indian/ Alaska Native	0	1	0	0	**1**
Asian	3	3	0	0	**6**
Native Hawaiian or Other Pacific Islander	1	0	0	0	**1**
Black or African American	7	7	1	1	**16**
White	14	18	3	3	**38**
More Than One Race	3	3	1	1	**8**
Total	**28**	**32**	**5**	**5**	**70**

13.3 *Analysis of Secondary Endpoints*

13.3.1 Statistical Analysis of Pharmacokinetics/Pharmacogenetics

Plasma drug concentrations and pharmacokinetic parameters will be presented in tabular and graphical form separately for each stratum. 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. Non-linear mixed effects models and non-compartmental methods will also be considered. Dose proportionality in pharmacokinetic parameters will also be investigated. As feasible, we will also investigate the associations between PK parameters and clinical and demographic variables such as toxicity, response, and PFS. We will also explore associations between PK parameters and number of prior treatments, age, body surface area, steroid use or concomitant medications

In addition to estimating individual pharmacokinetic parameters, we will also estimate the population parameters by taking into account both the inter- and intra-subject variability.

13.3.2 Pharmacodynamic Studies:

For cell-free DNA based assays where the intent is to determine whether K27 mutations can be detected in patients' blood or urine, we will summarize the percentage of patients in whom this mutation is detected within each stratum and at each time point. We will also numerically summarize changes across time within a patient.

For this study we will consider exploratory studies correlating these findings with various PK parameters as well as with PFS and OS, as feasible within each stratum.

13.3.3 Descriptive Efficacy Assessments

Another secondary aim of this study is to estimate the PFS and OS associated with panobinostat treatment. PFS will be measured from the time of treatment initiation until the time of progressive disease or death from any cause in patients with an event and until the time of last follow-up for patients who are progression free at the time of analysis. OS is measured similarly with the endpoint of death. We will also report any objective responses that have been observed along with duration of response as well as dose levels at which such responses were observed. Efficacy analyses will be conducted separately for each stratum.

References

1. Warren KE. Diffuse intrinsic pontine glioma: poised for progress. *Frontiers in oncology.* 2012;2:205.
2. Freeman CR, Farmer JP. Pediatric brain stem gliomas: a review. *Int J Radiat Oncol Biol Phys.* Jan 15 1998;40(2):265-271.
3. States) CCBTRotU. CBTRUS Statistical Report: Primary Brain and Central Nervous System Tumors Diagnosed in the United States in 2004 - 2006. . 2010.
4. Fisher PG, Breiter SN, Carson BS, et al. A clinicopathologic reappraisal of brain stem tumor classification. Identification of pilocystic astrocytoma and fibrillary astrocytoma as distinct entities. *Cancer.* Oct 1 2000;89(7):1569-1576.
5. Cohen KJ, Broniscer A, Glod J. Pediatric glial tumors. *Current treatment options in oncology.* Dec 2001;2(6):529-536.
6. Langmoen IA, Lundar T, Storm-Mathisen I, Lie SO, Hovind KH. Management of pediatric pontine gliomas. *Childs Nerv Syst.* Feb 1991;7(1):13-15.
7. Bouffet E, Raquin M, Doz F, et al. Radiotherapy followed by high dose busulfan and thiotepa. *Cancer.* 2000;88(3):685-692.
8. Cohen KJ, Heideman RL, Zhou T, et al. Temozolomide in the treatment of children with newly diagnosed diffuse intrinsic pontine gliomas: a report from the Children's Oncology Group. *Neuro-oncology.* 2011;13(4):410-416.
9. Dunkel I, O'Malley B, Finlay J. Is there a role for high-dose chemotherapy with stem cell rescue for brain stem tumors of childhood? *Pediatric neurosurgery.* 1996;24(5):263-266.
10. Finlay JL, August C, Packer R, et al. High-dose multi-agent chemotherapy followed by bone marrow 'rescue'for malignant astrocytomas of childhood and adolescence. *Journal of neuro-oncology.* 1990;9(3):239-248.
11. Hargrave D, Bartels U, Bouffet E. Diffuse brainstem glioma in children: critical review of clinical trials. *The Lancet Oncology.* 2006;7(3):241-248.
12. Jalali R, Raut N, Arora B, et al. Prospective evaluation of radiotherapy with concurrent and adjuvant temozolomide in children with newly diagnosed diffuse intrinsic pontine glioma. *International Journal of Radiation Oncology* Biology* Physics.* 2010;77(1):113-118.
13. Jansen M, Van Vuurden D, Vandertop W, Kaspers G. Diffuse intrinsic pontine gliomas: a systematic update on clinical trials and biology. *Cancer treatment reviews.* 2012;38(1):27-35.
14. Jennings MT, Sposto R, Boyett JM, et al. Preradiation chemotherapy in primary high-risk brainstem tumors: phase II study CCG-9941 of the Children's Cancer Group. *Journal of clinical oncology.* 2002;20(16):3431-3437.
15. Schwartzentruber J, Korshunov A, Liu XY, et al. Driver mutations in histone H3.3 and chromatin remodelling genes in paediatric glioblastoma. *Nature.* Feb 9 2012;482(7384):226-231.
16. Wu G, Broniscer A, McEachron TA, et al. Somatic histone H3 alterations in pediatric diffuse intrinsic pontine gliomas and non-brainstem glioblastomas. *Nat Genet.* Mar 2012;44(3):251-253.
17. Elsaesser SJ, Goldberg AD, Allis CD. New functions for an old variant: no substitute for histone H3.3. *Current opinion in genetics & development.* Apr 2010;20(2):110-117.
18. Skene PJ, Henikoff S. Histone variants in pluripotency and disease. *Development.* Jun

2013;140(12):2513-2524.

19. Buczkowicz P, Hoeman C, Rakopoulos P, et al. Genomic analysis of diffuse intrinsic pontine gliomas identifies three molecular subgroups and recurrent activating ACVR1 mutations. *Nat Genet.* May 2014;46(5):451-456.

20. Fontebasso AM, Papillon-Cavanagh S, Schwartzentruber J, et al. Recurrent somatic mutations in ACVR1 in pediatric midline high-grade astrocytoma. *Nat Genet.* May 2014;46(5):462-466.

21. Buczkowicz P, Hoeman C, Rakopoulos P, et al. Genomic analysis of diffuse intrinsic pontine gliomas identifies three molecular subgroups and recurrent activating ACVR1 mutations. *Nature genetics.* 2014;46(5):451-456.

22. Fontebasso AM, Papillon-Cavanagh S, Schwartzentruber J, et al. Recurrent somatic mutations in ACVR1 in pediatric midline high-grade astrocytoma. *Nature genetics.* 2014;46(5):462-466.

23. Taylor KR, Mackay A, Truffaux N, et al. Recurrent activating ACVR1 mutations in diffuse intrinsic pontine glioma. *Nature genetics.* 2014;46(5):457-461.

24. Project SJCsRHWUPCG. The genomic landscape of diffuse intrinsic pontine glioma and pediatric non-brainstem high-grade glioma. *Nature genetics.* 2014;46(5):444-450.

25. Jones C, Baker SJ. Unique genetic and epigenetic mechanisms driving paediatric diffuse high-grade glioma. *Nature Reviews Cancer.* 2014.

26. Maze I, Noh K-M, Soshnev AA, Allis CD. Every amino acid matters: essential contributions of histone variants to mammalian development and disease. *Nature Reviews Genetics.* 2014;15(4):259-271.

27. Yuen BT, Knoepfler PS. Histone H3. 3 mutations: a variant path to cancer. *Cancer Cell.* 2013;24(5):567-574.

28. Khuong-Quang D-A, Buczkowicz P, Rakopoulos P, et al. K27M mutation in histone H3. 3 defines clinically and biologically distinct subgroups of pediatric diffuse intrinsic pontine gliomas. *Acta neuropathologica.* 2012;124(3):439-447.

29. Schwartzentruber J, Korshunov A, Liu X-Y, et al. Driver mutations in histone H3. 3 and chromatin remodelling genes in paediatric glioblastoma. *Nature.* 2012;482(7384):226-231.

30. Lewis PW, Muller MM, Koletsky MS, et al. Inhibition of PRC2 activity by a gain-of-function H3 mutation found in pediatric glioblastoma. *Science.* May 17 2013;340(6134):857-861.

31. Simon JA, Lange CA. Roles of the EZH2 histone methyltransferase in cancer epigenetics. *Mutation research.* Dec 1 2008;647(1-2):21-29.

32. Venneti S, Garimella MT, Sullivan LM, et al. Evaluation of histone 3 lysine 27 trimethylation (H3K27me3) and enhancer of Zest 2 (EZH2) in pediatric glial and glioneuronal tumors shows decreased H3K27me3 in H3F3A K27M mutant glioblastomas. *Brain Pathol.* Sep 2013;23(5):558-564.

33. Chan KM, Fang D, Gan H, et al. The histone H3.3K27M mutation in pediatric glioma reprograms H3K27 methylation and gene expression. *Genes Dev.* May 1 2013;27(9):985-990.

34. Bender S, Tang Y, Lindroth AM, et al. Reduced H3K27me3 and DNA hypomethylation are major drivers of gene expression in K27M mutant pediatric high-grade gliomas. *Cancer Cell.* 2013;24(5):660-672.

35. Chan K-M, Fang D, Gan H, et al. The histone H3. 3K27M mutation in pediatric glioma

reprograms H3K27 methylation and gene expression. *Genes & development.* 2013;27(9):985-990.

36. Bender S, Tang Y, Lindroth AM, et al. Reduced H3K27me3 and DNA Hypomethylation Are Major Drivers of Gene Expression in K27M Mutant Pediatric High-Grade Gliomas. *Cancer Cell.* Nov 11 2013;24(5):660-672.

37. Yoshida M, Nomura S, Beppu T. Effects of trichostatins on differentiation of murine erythroleukemia cells. *Cancer research.* 1987;47(14):3688-3691.

38. Yoshida M, Beppu T. Reversible arrest of proliferation of rat 3Y1 fibroblasts in both the G1 and G2 phases by trichostatin A. *Experimental cell research.* 1988;177(1):122-131.

39. Itazaki H, Nagashima K, Sugita K, et al. Isolation and structural elucidation of new cyclotetrapeptides, trapoxins A and B, having detransformation activities as antitumor agents. *The Journal of antibiotics.* 1990;43(12):1524-1532.

40. YOSHIDA M, HOSHIKAWA Y, KOSEKI K, MORI K, BEPPU T. Structural specificity for biological activity of trichostatin A, a specific inhibitor of mammalian cell cycle with potent differentiation-inducing activity in Friend leukemia cells. *The Journal of antibiotics.* 1990;43(9):1101-1106.

41. Sugita K, Koizumi K, Yoshida H. Morphological reversion of sis-transformed NIH3T3 cells by trichostatin A. *Cancer research.* 1992;52(1):168-172.

42. Yoshida H, Sugita K. A Novel Tetracyclic Peptide, Trapoxin, Induces Phenotypic Change from Transformed to Normal in sis-Oncogene-transformed NIH3T3 Cells. *Cancer Science.* 1992;83(4):324-328.

43. Medina V, Edmonds B, Young GP, James R, Appleton S, Zalewski PD. Induction of caspase-3 protease activity and apoptosis by butyrate and trichostatin A (inhibitors of histone deacetylase): dependence on protein synthesis and synergy with a mitochondrial/cytochrome c-dependent pathway. *Cancer research.* 1997;57(17):3697-3707.

44. Biggs JR, Kudlow JE, Kraft AS. The role of the transcription factor Sp1 in regulating the expression of the WAF1/CIP1 gene in U937 leukemic cells. *Journal of Biological Chemistry.* 1996;271(2):901-906.

45. Nakano K, Mizuno T, Sowa Y, et al. Butyrate activates the WAF1/Cip1 gene promoter through Sp1 sites in a p53-negative human colon cancer cell line. *Journal of Biological Chemistry.* 1997;272(35):22199-22206.

46. Sowa Y, Orita T, Minamikawa S, et al. Histone deacetylase inhibitor activates the WAF1/Cip1 gene promoter through the Sp1 sites. *Biochemical and biophysical research communications.* 1997;241(1):142-150.

47. Sambucetti LC, Fischer DD, Zabludoff S, et al. Histone deacetylase inhibition selectively alters the activity and expression of cell cycle proteins leading to specific chromatin acetylation and antiproliferative effects. *Journal of Biological Chemistry.* 1999;274(49):34940-34947.

48. Yu X, Guo ZS, Marcu MG, et al. Modulation of p53, ErbB1, ErbB2, and Raf-1 expression in lung cancer cells by depsipeptide FR901228. *Journal of the National Cancer Institute.* 2002;94(7):504-513.

49. Fuino L, Bali P, Wittmann S, et al. Histone deacetylase inhibitor LAQ824 down-regulates Her-2 and sensitizes human breast cancer cells to trastuzumab, taxotere, gemcitabine, and epothilone B. *Molecular cancer therapeutics.* 2003;2(10):971-984.

50. Nimmanapalli R, Fuino L, Bali P, et al. Histone deacetylase inhibitor LAQ824 both

lowers expression and promotes proteasomal degradation of Bcr-Abl and induces apoptosis of imatinib mesylate-sensitive or-refractory chronic myelogenous leukemia-blast crisis cells. *Cancer research.* 2003;63(16):5126-5135.

51. Atadja P. Development of the pan-DAC inhibitor panobinostat (LBH589): successes and challenges. *Cancer letters.* 2009;280(2):233-241.

52. Ocio EM, Vilanova D, Atadja P, et al. In vitro and in vivo rationale for the triple combination of panobinostat (LBH589) and dexamethasone with either bortezomib or lenalidomide in multiple myeloma. *haematologica.* 2010;95(5):794-803.

53. Hideshima T, Richardson PG, Anderson KC. Mechanism of action of proteasome inhibitors and deacetylase inhibitors and the biological basis of synergy in multiple myeloma. *Molecular cancer therapeutics.* 2011;10(11):2034-2042.

54. Grasso CS, Tang Y, Truffaux N, et al. Functionally defined therapeutic targets in diffuse intrinsic pontine glioma. *Nature medicine.* 2015.

55. Broniscer A, Baker JN, Baker SJ, et al. Prospective collection of tissue samples at autopsy in children with diffuse intrinsic pontine glioma. *Cancer.* 2010;116(19):4632-4637.

56. Monje M, Mitra SS, Freret ME, et al. Hedgehog-responsive candidate cell of origin for diffuse intrinsic pontine glioma. *Proceedings of the National Academy of Sciences.* 2011;108(11):4453-4458.

57. Thirant C, Bessette B, Varlet P, et al. Clinical relevance of tumor cells with stem-like properties in pediatric brain tumors. *PloS one.* 2011;6(1):e16375.

58. Bishton MJ, Harrison SJ, Martin BP, et al. Deciphering the molecular and biologic processes that mediate histone deacetylase inhibitor–induced thrombocytopenia. *Blood.* 2011;117(13):3658-3668.

59. Giver C, Jaye D, Waller E, Kaufman J, Lonial S. Rapid recovery from panobinostat (LBH589)-induced thrombocytopenia in mice involves a rebound effect of bone marrow megakaryocytes. *Leukemia.* 2011;25(2):362-365.

60. Panobinostat Investigator's Brochure. Secura Bio Inc. Edition 17, 12-Mar-2020.

61. Lewis L, Frank R, Dandamudi U, et al. Influence of food on the pharmacokinetics (PK) of panobinostat (LBH589), an orally active histone deacetylase inhibitor, in patients with advanced cancer. Paper presented at: ASCO Annual Meeting Proceedings2009.

62. Clive S, Woo MM, Nydam T, Kelly L, Squier M, Kagan M. Characterizing the disposition, metabolism, and excretion of an orally active pan-deacetylase inhibitor, panobinostat, via trace radiolabeled 14C material in advanced cancer patients. *Cancer chemotherapy and pharmacology.* 2012;70(4):513-522.

63. Giles F, Fischer T, Cortes J, et al. A phase I study of intravenous LBH589, a novel cinnamic hydroxamic acid analogue histone deacetylase inhibitor, in patients with refractory hematologic malignancies. *Clin Cancer Res.* Aug 1 2006;12(15):4628-4635.

64. Shapiro GI, Frank R, Dandamudi UB, et al. The effect of food on the bioavailability of panobinostat, an orally active pan-histone deacetylase inhibitor, in patients with advanced cancer. *Cancer Chemother Pharmacol.* Feb 2012;69(2):555-562.

65. Abayomi OK. Pathogenesis of irradiation-induced cognitive dysfunction. *Acta Oncol.* 1996;35(6):659-663.

66. Fukutomi A, Hatake K, Matsui K, et al. A phase I study of oral panobinostat (LBH589) in Japanese patients with advanced solid tumors. *Investigational new drugs.* Jun 2012;30(3):1096-1106.

67. Drappatz J, Lee EQ, Hammond S, et al. Phase I study of panobinostat in combination with bevacizumab for recurrent high-grade glioma. *J Neurooncol.* Mar 2012;107(1):133-138.

68. Goldberg JM G-BJ, Sulis ML, Gardner RA, Pollard JA, Aquino V, Winick NJ, Bostrom BC, Fu C, Hutchinson RJ, Manley TJ, Mody R, Oesterheld J, Thomson B, JPark JR, Cassaer J, Eckroth E, Sposto R, Messinger YH, HiJiya N, Gaynon PS, Barredo JC. A Phase I Dose Finding Study of Panobinostat in Children with Hematologic Malignancies: Initial Report of TACL Study T2009-012 in Children with Acute Leukemia *ASH Annual Meeting.* Vol 124:3705. San Francisco, CA2014.

69. San-Miguel JF, Hungria VT, Yoon S-S, et al. Panobinostat plus bortezomib and dexamethasone versus placebo plus bortezomib and dexamethasone in patients with relapsed or relapsed and refractory multiple myeloma: a multicentre, randomised, double-blind phase 3 trial. *The Lancet Oncology.* 2014;15(11):1195-1206.

70. Novartis. Farydak panobinostat package insert. Revised 2/2015 ed2015.

71. Cheung YK. *Dose finding by the continual reassessment method*: CRC Press; 2011.

72. Lee SM, Cheung YK. Model calibration in the continual reassessment method. *Clinical Trials.* 2009;6(3):227-238.

73. Wages NA, Conaway MR, O'Quigley J. Performance of two-stage continual reassessment method relative to an optimal benchmark. *Clinical Trials.* 2013:1740774513503521.

74. Lee EQ, Reardon DA, Schiff D, et al. Phase II study of panobinostat in combination with bevacizumab for recurrent glioblastoma and anaplastic glioma. *Neuro Oncol*. 2015;17(6):862-7.

Appendix A: Performance Scales

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: Medications Which May Cause QTc Prolongation

The following table presents a list of drugs that may prolong the QTc. These drugs are prohibited during the study. Providers should consult a frequently-updated drug information reference for a complete list.

Table 21

Alfuzocin	Foscarnet	Ondansetron
Amantadine	Fosphenytoin	Pentamidine
Amiodarone (cordarone)	Gatifloxacin	Pimozide
Amitriptyline	Gemifloxacin	Procainamide
Arsenic trioxide	Grepafloxacin	Protiptyline
Azithromycin	Halofantrine	Quetiapine
Bepridil	Haloperidol	Quinidine
Chloral hydrate	Ibutilide	Quinine
Chloroquine	Imipramine	Risperidone
Chlorpromazine	Indapamide	Salmeterol
Cisapride	Isradipine	Sotalol
Clarithromycin	Levofloxacin	Sparfloxacin
Cloroquine	Levomethadyl	Sumatriptan
Desipramine	Lithium	Tacrolimus
Disopyramide	Mesoridazine	Tamoxifen
Dofetilide	Methadone	Telithromycin
Dolesetron	Moexipril/HCTZ	Thioridazine
Domperidone	Moxifloxacin	Tizanidine
Doxepin	Naratriptan	Vardenifil
Droperidol	Nicardipine	Venlaflaxine
Erythromycin	Nortriptyline	Voriconizole
Felbamate	Octreotide	Ziprasidone
Flecainide	Ofloxacin	Zolmitriptan

Appendix C: Partial List of Common Moderate and Strong Inhibitors of CYP3A4, and Common Sensitive CYP2D6 Substrates

This appendix is a partial list of common moderate to strong inhibitors of CYP3A4, and common sensitive CYP2D6 substrates. Providers should consult a frequently-updated drug information reference for a complete list.

Table 22

Common Moderate and Strong Inhibitors of CYP3A4	
HIV Antivirals:	cimetidine
indinavir	amiodarone
nelfinavir	NOT azithromycin
ritonavir	chloramphenicol
clarithromycin	ciprofloxacin
itraconazole	delaviridine
ketoconazole	diethyldithiocarbamate
nefazodone	fluvoxamine
saquinavir	gestodene
telithromycin	imatinib
aprepitant	mibefradil
erythromycin	mifepristone
fluconazole	norfloxacin
grapefruit juice	norfluoxetine
verapamil	star fruit
diltiazem	voriconazole

Table 23

Common Sensitive CYP2D6 Substrates	
Beta Blockers:	dexfenfluramine
carvedilol	dextromethorphan
S-metoprolol	duloxetine
propafenone	encainide
timolol	flecainide
Antidepressants:	fluoxetine
amitriptyline	fluvoxamine
clomipramine	lidocaine
desipramine	metoclopramide
imipramine	methoxyamphetamine
paroxetine	mexilletine
Antipsychotics:	minaprine
haloperidol	nebivolol

perphenazine risperidone→9OH thioridazine zuclopenthixol alprenolol amphetamine aripiprazole atomoxetine bufuralol chlorpheniramine chlorpromazine codeine (→O-desMe) debrisoquine	nortriptyline ondansetron oxycodone perhexiline phenacetin phenformin promethazine propranolol sparteine tamoxifen tramadol venlafaxine

Appendix D: Dosing Tables

5 mg/m² *(Patients with BSAs <0.8m² are not eligible to be treated at this dose)*					
Min BSA (m²)	**Max BSA (m²)**	**Daily panobinostat Dose (mg)**	**Min BSA (m²)**	**Max BSA (m²)**	**Daily panobinostat Dose (mg)**
0.80	1.49	5	1.50	2.5	10
10 mg/m² *(Patients with BSAs <0.65m² are not eligible to be treated at this dose)*					
Min BSA (m²)	**Max BSA (m²)**	**Daily panobinostat Dose (mg)**	**Min BSA (m²)**	**Max BSA (m²)**	**Daily panobinostat Dose (mg)**
0.65	0.78	5	1.75	2.25	20
0.79	1.25	10	2.26	2.50	25
1.26	1.74	15			
16 mg/m² *(Patients with BSAs <0.65m² are not eligible to be treated at this dose)*					
Min BSA (m²)	**Max BSA (m²)**	**Daily panobinostat Dose (mg)**	**Min BSA (m²)**	**Max BSA (m²)**	**Daily panobinostat Dose (mg)**
0.65	0.78	10	1.72	2.03	30
0.79	1.09	15	2.04	2.34	35
1.10	1.40	20	2.35	2.5	40
1.41	1.71	25			
22 mg/m² *(Patients with BSAs <0.65m² are not eligible to be treated at this dose)*					
Min BSA (m²)	**Max BSA (m²)**	**Daily panobinostat Dose (mg)**	**Min BSA (m²)**	**Max BSA (m²)**	**Daily panobinostat Dose (mg)**
0.65	0.78	15	1.71	1.93	40
0.79	1.02	20	1.94	2.15	45
1.03	1.24	25	2.16	2.38	50
1.25	1.47	30	2.39	2.50	55
1.48	1.70	35			
28 mg/m² *(Patients with BSAs <0.50m² are not eligible to be treated at this dose)*					
Min BSA (m²)	**Max BSA (m²)**	**Daily panobinostat**	**Min BSA (m²)**	**Max BSA (m²)**	**Daily panobinostat**

		Dose (mg)			**Dose (mg)**
0.50	0.62	15	1.52	1.69	45
0.63	0.78	20	1.70	1.87	50
0.79	0.98	25	1.88	2.05	55
0.99	1.16	30	2.06	2.23	60
1.17	1.33	35	2.24	2.41	65
1.34	1.51	40	2.42	2.50	70
36 mg/m^2 *(Patients with BSAs <0.50m^2 are not eligible to be treated at this dose)*					
Min BSA (m^2)	**Max BSA (m^2)**	**Daily panobinostat Dose (mg)**	**Min BSA (m^2)**	**Max BSA (m^2)**	**Daily panobinostat Dose (mg)**
0.5	0.62	20	1.60	1.73	60
0.63	0.76	25	1.74	1.87	65
0.77	0.90	30	1.88	2.01	70
0.91	1.04	35	2.02	2.15	75
1.05	1.18	40	2.16	2.29	80
1.19	1.31	45	2.30	2.43	85
1.32	1.45	50	2.44	2.50	90
1.46	1.59	55			

Appendix E: Patient Diary

Protocol: PBTC-047, **Stratum 1** Drug: Panobinostat

Patient Name: _____

Course#: _____ BSA: _____ Dose: _____ mg/m^2/day

Start Date: ____/____/_____ End Date: ____/____/_____

Patients are encouraged to take their dose of panobinostat at the same time each day, preferably in the morning. Each dose of panobinostat should be taken with a 4 oz / 120 ml glass of water. Drug must be taken on an empty stomach (either 1 hour before or after meals) on Course 1, Day 1 (1st dose) and Day 3 (2nd dose) and may be taken with or without food for the remaining doses. Patients should be instructed to swallow the capsules whole and not chew them. Patients must avoid grapefruit or grapefruit juice and Seville (sour) oranges during the entire study.

If the patient forgets to take his/her dose during the morning on a scheduled treatment day, then he/she should take panobinostat on that same day within 12 hours after the missed dose if possible. After more than 12 hours, that day's dose should be withheld, and the patient should wait to take panobinostat until the next scheduled treatment day (i.e., patients should be instructed not to try to make-up the missed dose after 12 hours). The patient should then continue treatment with the original dosing schedule. If vomiting occurs within 15 minutes, the dose should be repeated. If a dose is missed/ unable to be given, record on the patient diary and continue counting the days of the course, with treatment administered on originally scheduled days.

EXAMPLE	**Date**	**Time**		**Total dose taken**	Comments
Day 1	*1 / 15 / 15*	*8 : 30*	*AM PM*	*15 mg*	*Example*: He felt nauseated an hour after taking the drug, but did not vomit.
	Date	**Time**		**Total dose taken**	**Comments**
1st Week of Course		:	AM PM		
Dose 1		:	AM PM		
Dose 2		:	AM PM		
Dose 3		:	AM PM		
2nd Week of Course		:	AM PM		
Dose 4		:	AM PM		
Dose 5		:	AM PM		
Dose 6		:	AM PM		
3rd Week of Course		:	AM PM		
Dose 7		:	AM PM		
Dose 8		:	AM PM		
Dose 9		:	AM PM		
4th Week of Course	Rest, No Drug				

Protocol: PBTC-047, **Stratum 2** Drug: Panobinostat

Patient Name: _____

Course#: _____ BSA: _____ Dose: _____ mg/m^2/day

Start Date: ____/____/_____ End Date: ____/____/_____

Patients are encouraged to take their dose of panobinostat at the same time each day, preferably in the morning. Each dose of panobinostat should be taken with a 4 oz / 120 ml glass of water. Drug must be taken on an empty stomach (either 1 hour before or after meals) on Course 1, Day 1 (1st dose) and Day 3 (2nd dose) and may be taken with or without food for the remaining doses. Patients should be instructed to swallow the capsules whole and not chew them. Patients must avoid grapefruit or grapefruit juice and Seville (sour) oranges during the entire study.

If the patient forgets to take his/her dose during the morning on a scheduled treatment day, then he/she should take panobinostat on that same day within 12 hours after the missed dose if possible. After more than 12 hours, that day's dose should be withheld, and the patient should wait to take panobinostat until the next scheduled treatment day (i.e., patients should be instructed not to try to make-up the missed dose after 12 hours). The patient should then continue treatment with the original dosing schedule. If vomiting occurs within 15 minutes, the dose should be repeated. If a dose is missed/ unable to be given, record on the patient diary and continue counting the days of the course, with treatment administered on originally scheduled days.

EXAMPLE	**Date**	**Time**		**Total dose taken**	Comments
Day 1	*1 / 15 / 15*	*8 : 30*	*AM PM*	*15 mg*	*Example*: He felt nauseated an hour after taking the drug, but did not vomit.

	Date	**Time**		**Total dose taken**	**Comments**
1st Week of Course					
Dose 1		:	AM PM		
Dose 2		:	AM PM		
Dose 3		:	AM PM		
2nd Week of Course	Rest, No Drug				
3rd Week of Course					
Dose 4		:	AM PM		
Dose 5		:	AM PM		
Dose 6		:	AM PM		
4th Week of Course	Rest, No Drug				