A Feasibility Study Examining the Use of Non-Invasive Focused Ultrasound (FUS) with oral Panobinostat Administration in Children with Progressive Diffuse Midline Glioma (DMG)

Short Title: FUS Panobinostat for DMG
Columbia University Irving Medical Center

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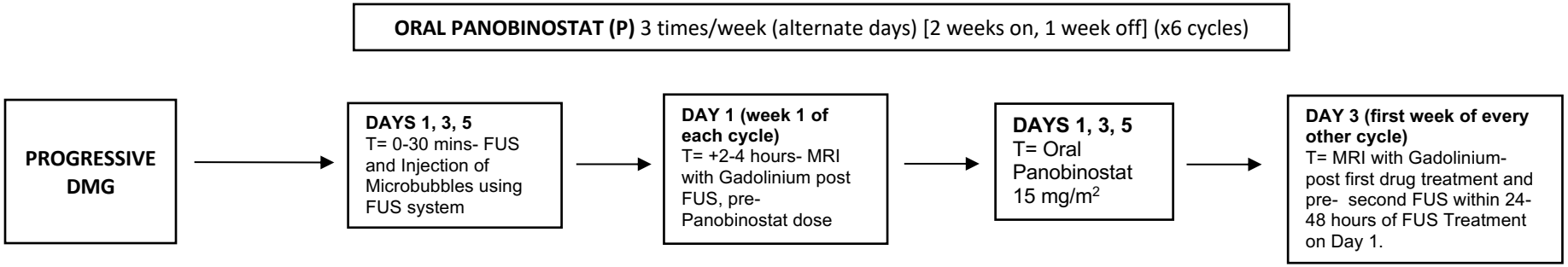
Trial Title	**Feasibility Study Examining the use of Non-Invasive Focused Ultrasound (FUS) with oral Panobinostat Administration in Children with progressive Diffuse Midline Glioma (DMG).**	
Short Title	FUS Panobinostat for DMG	
Trial Design	Prospective non-randomized single arm feasibility study	
Trial Participants	Children	
Planned Sample Size	Maximum 15 subjects	
Treatment duration	6 months	
Follow-up duration	2 years	
Investigational Medical Device: Focused Ultrasound		
	Objectives	Outcome measures
Primary Endpoint	To evaluate the safety and feasibility of using FUS to open one, two or three tumor sites for administration of Panobinostat in children with progressive DMG	Physical exam, laboratory studies,
Secondary Endpoints	To determine blood brain barrier/tumor imaging changes using FUS in children with progressive DMG	Documenting changes in the pattern of contrast enhancement on MRI
	To evaluate the 6 month Progression Free Survival and 6 month Overall Survival	Calculate 6 month Progression Free Survival and 6 month Overall Survival

SCHEMATIC OVERVIEW OF TREATMENT PLAN FOLLOWING A 3+3 NOTS** ESCALATION DESIGN*#



*** - A number of tumor sites (NOTS) Escalation Schema is laid out in Section 7.2 and 7.4.**
#- Checkpoint MRIs (without contrast) will be conducted as laid out in NOTS escalation schema prior to each NOTS escalation determination stage.
****- Number of Tumor Sites (NOTS)**

PROTOCOL SIGNATURE PAGE

I confirm that I have read this protocol, I understand it, and I will work according to this protocol and to the ethical principles stated in the latest version of the Declaration of Helsinki, the applicable ICH guidelines for good clinical practices, and the applicable federal, state, and local laws, rules, and regulations relating to the conduct of the protocol. I have read and understand the information in the Investigators' Brochure (or Manufacturer's Brochure) regarding the risks and potential benefits. I will promptly submit the protocol to the applicable IRB for review and approval. Once the protocol has been approved by the IRB, I understand that any modification made during the course of the study must first be approved by the IRB, prior to implementation except when such modification is made to remove an immediate hazard to the subject. I certify that I, and the study staff, have received the requisite training to conduct this research protocol. I agree to maintain adequate and accurate records in accordance with Columbia University Irving Medical Center (CUIMC), and Herbert Irving Cancer Center policies, federal, state and local laws and regulations. I agree to maintain the confidentiality of all information received or developed in connection with this protocol.

Instructions to Principal Investigator: Sign and Date

_____ _____

Signature of Principal Investigator Date

_____

Principal Investigator Name (Print)

_____

Name of Institution

FUNDING AND SUPPORT IN KIND:

REGULATORY SPONSOR:	**Cheng-Chia Wu, M.D.** 161 Fort Washington Avenue, 7th Floor New York, NY 10032 T: (212) 305-9005 F: (212) 305-5848 sz2764@cumc.columbia.edu
FUNDING SOURCE:	**Focused Ultrasound Foundation** 1230 Cedars Court, Suite 206 Charlottesville, VA 22903 T: (434) 220-4993 F: (434) 220-4978 Info@fusfoundation.org
Study Agent:	Oral Panobinostat
IND Status:	**IND #: 152942** **IND Sponsor:** Cheng-Chia Wu, M.D.

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1. STUDY PROTOCOL

1.1 Introduction

This document is a protocol for a human research study. This study is going to be conducted according to U.S. and international standards of Good Clinical Practice (FDA Title 21 part 312 and International Conference on Harmonization guidelines), applicable government regulations, and Columbia University Irving Medical Center institutional research policies and procedures.

1.2 Investigational Products

Device name: Focused Ultrasound by Sonic Concepts
Device description: A single-element, spherical-segment FUS transducer (H-231, Sonic Concepts, Bothell, WA) driven by a function generator (Agilent, Palo Alto, CA, USA) through a 50-dB power amplifier (E&I, Rochester, NY, USA). The confocally mounted hydrophone (Y107, Sonic Concepts, WA, USA) is driven by a preamplifier (Olympus, Waltham, MA, USA).
Device Model/Version #: H-231

Device name: Neuronavigation System by Rogue Research
Device description: This device will be used for monitoring the procedure.
Device Model/Version #: Brainsight system 2.3.3

Drug name: Definity
Manufacturer: Lantheus Medical Imaging
Dose: 10 µl/kg
Route of administration: Intravenous
This drug is FDA-approved (NDA 21-064) but not being used in accordance with labeling.

Drug name: Oral Panobinostat
Dose: 15 mg/m^2
Route of administration: Oral
This drug is FDA-approved but not being used in accordance with labeling.

2. BACKGROUND AND RATIONALE

2.1 Diffuse Midline Gliomas

Diffuse midline gliomas (DMGs), formerly known as diffuse intrinsic pontine gliomas (DIPG) and thalamic gliomas, constitute 10% of all pediatric central nervous system (CNS) tumors. DMG is a WHO grade IV glioma that arises in the pons, thalamus and other midline structures. The most common type is a diffusely infiltrating astrocytoma (diffuse intrinsic pontine glioma – DIPG). Subjects with DIPG have a poor prognosis - median survival is usually reported to be 9 months, and nearly 90% of children die within 18 months from diagnosis [1-11]. In the molecular era, biopsies for DIPG are considered standard of care (SOC) for diagnostic purposes. The mainstay of treatment is radiation, typically to a dose of 54 Gy to the primary tumor site. No benefit from doses up to 78 Gy has been seen [8]. Surgical resection does not influence outcome [12] and is often not feasible in this extremely eloquent part of the central nervous system. Numerous

studies of systemic chemotherapy have all failed to demonstrate any significant improvement in survival, despite at times using very intensive chemotherapy regimens. This repeated failure of systemic therapy is, in part, due to an intact blood brain barrier (BBB), which limits drug access to the tumor.

2.2 Preclinical studies using FUS with microbubbles

Focused ultrasound (FUS) has been extensively studied in safely opening the blood-brain barrier (BBB) of various animal species and different disease-mimicking models. The application of FUS in conjunction with preformed intravenously administered microbubbles (i.e., DEFINITY®) produces bio-effects, which are confined only within the targeted region. Multiple studies have demonstrated that this targeted drug delivery system can be successfully applied in preclinical studies on rodents, rabbits, and non-human primates (NHP). This technique has shown efficacy in safely delivering a wide range of drugs such as contrast agents, sugars, antibodies, viruses, chemotherapeutics and neurotrophic factors into the brain [13-31]. The safety of opening the blood brain barrier using focused ultrasound was further proved in a murine pre-clinical study conducted by Englander et. al. that aimed to determine the safety and feasibility of this technique in a murine pontine glioma model. In this study mice were randomly assigned to control, FUS and double-FUS groups. Pulse and respiratory rate were continuously monitored during treatment. BBB opening was confirmed with gadolinium-enhanced MRI and Evans blue. The study showed that FUS successfully caused BBB opening while preserving normal cardiorespiratory and motor function.

Furthermore, FUS-mediated BBB opening has been shown to effectively decrease the amyloid plaque load and restore memory in rodents with Alzheimer's disease (AD), even without the administration of a drug [22]. These rodents were tested behaviorally and showed significant memory improvement following FUS treatment.

DEFINITY® Microbubbles are gas-core, lipid-shelled particles with diameters between 1-10 µm [32]. Their minute size allows them to circulate throughout the vasculature. DEFINITY® Microbubbles are compressible; thus, they respond to the alternating phases of an ultrasound wave by expanding and contracting, a response known as acoustic cavitation. These volumetric oscillations exert stresses onto the surrounding vascular walls and temporarily open the tight junctions that formulate the BBB. The magnitude and distribution of this bio effect can be controlled by adjusting the sonication parameters but is typically constrained within the center of the focal volume (Figure 1).

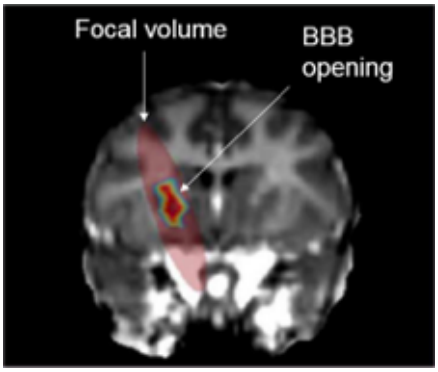


Figure 1: BBB opening within the targeted area of an NHP brain. The induced bioeffect is constrained within the center of the focal volume. Adapted from Karakatsani et al, 2017.

Dr. Konofagou's group and others have published extensive work confirming the accuracy and safety of this technique performed both in rodents, in NHP and, more recently, is being investigated in humans. Dr. Konofagou's research has recently shown that safe BBB opening can be achieved in an NHP model using clinically relevant acoustic pressures (i.e., mechanical index (MI) of 0.4) and the clinically recommended dose for imaging applications of DEFINITY® microbubbles, which are the parameters proposed for this clinical trial. Therefore, Dr. Konofagou et al has established that this technique is able to safely open the BBB and achieve targeted drug delivery in the brain using acoustic intensities far below the FDA limits for ultrasound imaging and drug doses equal to those routinely used in the clinic. In a study by Dr. Konofagou, the integrity of the BBB was restored within hours, depending on the selected parameters, e.g. the acoustic pressure or pulse length [27]. Permeability and volume of opening were both found to increase with the acoustic pressure and pulse length, but closing occurred within 48 to 72 hours in these cases. BBB can be safely and reversibly opened using focused ultrasound and DEFINITY® microbubbles, and that its integrity is restored within 2 days in rodents and between 2 and 4 days in NHPs.

All of Dr. Konofagou's nonclinical laboratory studies have been conducted in compliance with applicable requirements in the Good Laboratory Practice (GLP) regulations in 21 CRF Part 58 (See Appendix attached 5.0).

Dr. Konofagou's lab has recently developed a new clinical system for BBB opening applications in humans (Figure 2). Please see section 5 for a detailed description of the FUS device. The new system consists of two main parts: (i) ultrasound unit for sonication and real-time passive acoustic monitoring and (ii) neuro-navigator providing real-time guidance. In contrast to MR-guided FUS which requires sonication within an MRI magnet, this approach is based on an open-space, neuro-navigator-controlled sonication. The real-time guidance is achieved by registration of the physical space to the preloaded MRI that is displayed on the screen. The registration is achieved by the infrared camera (i.e. position sensor) reflecting the position of the beads secured close to the brain. The transducer is attached on the robotic arm and the position varies depending on the structure that will be sonicated. The transducer carries beads as well so its relative position to the brain can be seen on the screen. Once the transducer is locked at the

position of interest, the sonication starts. Real-time monitoring of the sonication by passive cavitation detection can be used to address the efficacy and safety of the technique. The sonication procedure coupled with the neuronavigation system has been tested on sitting, awake primates and the resulting BBB opening was similar to the results obtained from our previous equipment [19, 24, 25, 33-36].

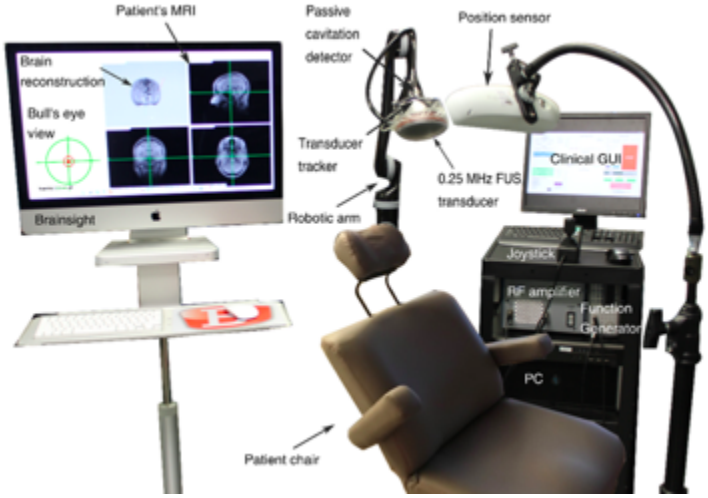


Figure 2: Clinical setup for BBB opening in pediatric patients.

2.3 Human Clinical Studies using FUS

There are several ongoing clinical trials in Canada, France and USA regarding the use of FUS and DEFINITY® microbubbles for BBB opening [13, 23, 37-39].

Preliminary results indicate that the use of FUS for the treatment of glioblastoma multiforme (GBM) and Alzheimer's disease (AD) is both safe and efficient in humans (Focused Ultrasound Foundation 2017). The first published clinical study regarding the application of FUS to increase BBB permeability was the one by Carpentier et al. [13]. The authors used an implantable unfocused single-element transducer called "SonoCloud", which was fixed in the skull bone and connected to an external power supply via a transdermal needle. GBM patients were enrolled in this study and were exposed to repeated monthly FUS treatments before receiving systemic chemotherapy with Carboplatin. The results showed that BBB was disrupted at acoustic pressures of up to 1.1 MPa (i.e., MI: 1.07) without detectable adverse effects on MRI or clinical examination. This trial was designed as a "dose escalation study", in which the acoustic pressure was increased from 0.5 to 1.1 MPa through five different "dose" levels (0.5, 0.65, 0.8, 0.95, 1.1 MPa or MI of 0.49, 0.63, 0.78, 0.93, 1.07 respectively). BBB disruption was correlated with the acoustic pressure amplitude. For the five different pressures used, the BBB opening incidence was 0/3, 0/6, 8/11, 6/7, and 14/14 respectively. Regarding safety outcomes, there was no evidence of acute hemorrhage, ischemia, or edema in immediate post-sonication, susceptibility-weighted angiography (SWAN), diffusion, or FLAIR sequences. Additionally, there was no FUS treatment-related clinical symptom or any report of unusual sensations during the sonication duration.

Recently the same group [40] reported an updated study where patients were implanted with a 1 MHz, 11.5-mm diameter cranial ultrasound device (SonoCloud-1, CarThera, Paris, France). The

device was activated monthly to transiently disrupt the BBB before intravenous (IV) Carboplatin chemotherapy (Area under the Curve 5 dosing). Nineteen patients received at least one sonication. In 65 US sessions, BBB disruption was visible on T1w MRI for 52 sonications. Treatment-related adverse events observed were transient and manageable: a transient edema at H1 and at D15. No Carboplatin-related neurotoxicity was observed. Patients with no or poor BBB disruption visible on MRI had a median Progression-Free Survival (PFS) of 2.73 months, and a median OS of 8.64 months. Patients with clear BBB disruption had a median PFS of 4.11 months and a median OS of 12.94 months.

A research group in Sunnybrook Research Institute follows another approach, where the generation of FUS is achieved through a 1024-element Insightec® ultrasonic array embedded within an MRI magnet. The multi-element hemi-spherical transducer called "ExAblate Neuro" has been approved by the FDA for MR-guided non-invasive thalamotomy for treatment of essential tremor [41]. Transcranial FUS application is possible through the electronic steering of the ultrasound beam and allows the capability of MR guidance. The first study showing safe, efficient, localized, reversible, and repeatable BBB opening in 5 AD patients using the ExAblate system was recently published [23]. AD patients received FUS treatments aimed at the dorsolateral prefrontal cortex. The size of the targeted area was 9×9 mm^2, achieved through a 3×3 grid multiple sonication design. BBB opening was fully reversible, with no contrast enhancement detected 1 day after treatment. Furthermore, there was no clinically significant worsening in the cognitive scores and no detectable reduction in beta-amyloid levels measured by ^{18}F-PET post-sonication.

Mainprize et al [37] used Non-invasive MR-Guided Focused Ultrasound (MRgFUS) to demonstrate safety and feasibility of MRgFUS BBB opening for 24 hours with systemically administered chemotherapy in patients with glioma in a phase I, single-arm, and open-label study. Five patients with previously confirmed or suspected high-grade glioma based on imaging underwent the MRgFUS in conjunction with administration of chemotherapy (n = 1 liposomal doxorubicin, n = 4 temozolomide) one day prior to their scheduled surgical resection. Samples of "sonicated" and "unsonicated" tissue were measured for the chemotherapy by liquid-chromatography-mass spectrometry. Complete follow-up was three months. The procedure was well tolerated, with no adverse clinical or radiologic events related to the procedure. The BBB within the target volume showed radiographic evidence of opening with an immediate 15–50% increased contrast enhancement on T1-weighted MRI, and resolution approximately 20 hours after.

2.4 Panobinostat

DNA and histones provide the main building blocks for nucleosomes, the structural units of chromatin that are important for packaging DNA. Changes in the structural configuration of chromatin to a relatively active (open) or inactive (condensed) form alters the accessibility of DNA for transcription, ultimately affecting gene expression. One of the major ways that transcription factor binding to DNA is regulated is through changes in chromatin conformation, which in turn is governed by chemical modifications such as the acetylation and deacetylation of lysine residues of core nucleosomal histones. These changes are under the control of the

opposing activities of histone deacetylase (HDAC) and histone acetylase (HAT), and lead to altered gene expression, including genes involved in cell cycle regulation, differentiation and apoptosis. Acetylation is generally linked to an 'open' chromatin state that is ready for transcription or that corresponds to actively transcribed genomic regions, whereas deacetylation is associated with a closed or inactive state, leading to gene repression. The relative degree of histone acetylation and deacetylation therefore controls the level at which a gene is transcribed. HDAC also has crucial roles in cell cycle proliferation and apoptosis, including transcription factors such as p53, NF-κB and E2F1, which play key roles in tumorigenesis and anti-tumor response, as well as proteins that do not directly regulate gene expression but instead regulate DNA repair (Ku70), the cellular cytoskeleton (α-tubulin) and protein stabilization (Hsp90). Notably, among non-histone HDAC substrates, Hsp90 plays a major role in the proper folding and stability of several major oncoproteins. HDAC activity also regulates cell protein turnover via the aggresome pathway, which if disrupted, results in the accumulation of polyubiquitinated misfolded protein aggregates, leading to cell stress and caspase-dependent apoptosis. These observations have extended the mechanism of anti-tumor activity of Panobinostat and other HDAC inhibitors to include effects on non-histone proteins, implicated in multiple oncogenic pathways, in conjunction with epigenetic changes [42].

Using brain tumor samples collected from children in the United States and Europe, Grasso et al. found that the drug Panobinostat and similar gene-regulating drugs may be effective at treating DIPG [43]. Using DIPG cell cultures from 16 patients, Grasso et al screened 83 existing and potential cancer drugs and found that histone deacetylase (HDAC) inhibitors consistently slowed cancer cell growth in DIPG cell lines, and inhibited DIPG growth and extended survival in a DIPG mouse model. Approximately 80 percent of DIPG tumors have a specific mutation in a histone gene, H3K27M, which blocks the ability of methyltransferase to methylate histones. The pan-HDAC inhibitor, Panobinostat, was the most effective at slowing the growth of DIPG cell lines that had the mutation, as well as those that did not. Low doses however were ineffective and resistance developed in H3K27M mutant tumors. Also Grasso *et al*. reported significant reduction in H3K27M mutant orthotopic tumor growth after just one administration of Panobinostat via CED. Hennika et al. have shown that intense regimen with high daily doses are most likely required, and that pharmacodynamics analysis showed increased levels of H3 acetylation in tumor tissue demonstrating target inhibition at higher dose (20 mg/kg) schedule [44].

Panobinostat (Farydak™) is currently being investigated in late phase clinical trials for adults with T cell lymphoma and has recently been approved in the US and EU for the use in adults with relapsed refractory myeloma. Systemic (oral) administration of Panobinostat at effective doses in hematological malignancies, however, has been associated with significant toxicity – such as thrombocytopenia and cardiac toxicity. There is no clinical evidence that Panobinostat crosses the blood brain barrier when given systemically at tolerated doses. Also, higher doses of Panobinostat when given systemically were toxic in preclinical studies. It has been used to reactivate latent HIV in adult patients to facilitate the therapeutic effect of antiretroviral therapy to some effect. In these studies, patients had serial CSF sampled by lumbar puncture over a 2-week period following oral Panobinostat administration. Panobinostat was not detectable in the CSF of these patients at any time point [45].

The fact that Panobinostat seems to be the most efficacious clinically available drug against DIPG cells, that effective oral dosing is likely to cause unacceptable systemic toxicity and that it does not penetrate the CNS makes it of considerable interest as a treatment for DIPG by FUS.

3. DRUG INFORMATION

3.1 Panobinostat

3.1.1 Source and Pharmacology

Panobinostat or its commercially available oral form (FARYDAK) is a deacetylase (DAC) inhibitor. DACs, also known as histone DACs (HDAC), are responsible for regulating the acetylation of about 1750 proteins in the body; their functions are involved in many biological processes including DNA replication and repair, chromatin remodeling, transcription of genes, progression of the cell-cycle, protein degradation and cytoskeletal reorganization. In multiple myeloma, there is an overexpression of DAC proteins. Panobinostat inhibits class I (HDACs 1, 2, 3, 8), class II (HDACs 4, 5, 6, 7, 9, 10) and class IV (HDAC 11) proteins. Panobinostat's antitumor activity is believed to be attributed to epigenetic modulation of gene expression and inhibition of protein metabolism. Panobinostat also exhibits cytotoxic synergy with bortezomib, a proteasome inhibitor concurrently used in treatment of multiple myeloma.

3.1.2 Formulation and Stability

Panobinostat lactate anhydrous is both chemically and thermodynamically a stable crystalline form with no polymorphic behavior. Panobinostat free base is not chiral and shows no specific optical rotation. Panobinostat lactate anhydrous is slightly soluble in water. Solubility of Panobinostat lactate anhydrous is pH-dependent, with the highest solubility in buffer pH 3.0 (citrate).

FARYDAK capsules contain 10 mg, 15 mg, or 20 mg Panobinostat free base (equivalent to 12.58 mg, 18.86 mg, and 25.15 mg respectively of Panobinostat lactate). The inactive ingredients are magnesium stearate, mannitol, microcrystalline cellulose and pregelatinized starch. The capsules contain gelatin, FD&C Blue 1 (10 mg capsules), yellow iron oxide (10 mg and 15 mg capsules), red iron oxide (15 mg and 20 mg capsules) and titanium dioxide.

3.1.3 Guidelines for Administration

Panobinostat (FARYDAK) should be taken orally once on each scheduled day at about the same time, either with or without food.

Panobinostat capsules should be swallowed whole with a cup of water. Do not open, crush, or chew the capsules.

If a dose is missed it can be taken up to 12 hours after the specified dose time. If vomiting occurs the patient should not repeat the dose but should take the next usual scheduled dose.

3.1.4 Risks Associated with Panobinostat

The most common adverse reactions (incidence of at least 20%) in clinical studies are diarrhea, fatigue, nausea, peripheral edema, decreased appetite, pyrexia, and vomiting.

The most common non-hematologic laboratory abnormalities (incidence ≥ 40%) are: Hypophosphatemia, hypokalemia, hyponatremia, and increased creatinine.

The most common hematologic laboratory abnormalities (incidence ≥ 60%) are: thrombocytopenia, lymphopenia, leukopenia, neutropenia, and anemia.

Panobinostat carries a boxed warning alerting patients and health care professionals that severe diarrhea and severe and fatal cardiac events, arrhythmias and electrocardiogram (ECG) changes have occurred in patients receiving Panobinostat.

Other notable adverse drug reactions of Panobinostat not described above, which were either clinically significant, or occurred with a frequency less than 10% but had a frequency in the Panobinostat arm greater than 2% over the control arm in the multiple myeloma clinical trial are listed below:

Infections and infestations: hepatitis B.
Endocrine disorders: hypothyroidism.
Metabolism and nutrition disorders: hyperglycemia, dehydration, fluid retention, hyperuricemia, hypomagnesemia.
Nervous system disorders: dizziness, headache, syncope, tremor, dysgeusia.
Cardiac disorders: palpitations.
Vascular disorders: hypotension, hypertension, orthostatic hypotension.
Respiratory, thoracic and mediastinal disorders: cough, dyspnea, respiratory failure, rales, wheezing.
Gastrointestinal disorders: abdominal pain, dyspepsia, gastritis, cheilitis, abdominal distension, dry mouth, flatulence, colitis, gastrointestinal pain.
Skin and subcutaneous disorders: skin lesions, rash, erythema.
Musculoskeletal and connective tissue disorders: joint swelling.
Renal and urinary disorders: renal failure, urinary incontinence.
General disorders and administration site conditions: chills.
Investigations: blood urea increased, glomerular filtration rate decreased, blood alkaline phosphatase increased.

3.2 Gadolinium

3.2.1 Source and Pharmacology

Gadolinium based contrast agents are derived from Gadolinium is an element with atomic symbol Gd, atomic number 64, and atomic weight 157.25.

3.2.2 Formulation and Stability

Storage requirement

The original gadolinium solution will be stored in the Radiology at a temperature of 20- 25° C as part of standard procedures.

3.2.3 Guidelines for Administration

Gadolinium-based MRI contrast agent is administered intravenously in approximately, 0.2 mL/kg (0.1 mmoles/ kg) at a rate of 10 mL per 15 seconds.

3.2.4 Potential adverse effects

IV administered gadolinium-based contrast agents have been identified as the cause of the rare disease nephrogenic systemic fibrosis (NSF), which presents as acute to subacute edema of the extremities as well as cutaneous plaques[46]. NSF can also be associated with extremity pain and muscle contractures and can lead to severe disability and even death. Development of NSF after gadolinium administration has been linked to renal impairment, with prevalence of 2-6% in patients with severe renal dysfunction[46]. Nevertheless, patients in this study will be screened for appropriate renal function. As with any medication, there is risk of allergic reaction and possible anaphylaxis with administration of gadolinium. Patient's will be monitored for allergic reactions and treated appropriately if any evidence of allergy is identified. Patient's with known pre-existing contrast allergies will be excluded from participation in the study.

4. MICROBUBBLES

4.1 General Information

DEFINITY® microbubbles are FDA-approved for use in adults (NDA 21-064) and are routinely used in ultrasound imaging applications, such as echocardiography or renal imaging, for over 15 years. In the proposed study, we will use the clinically recommended dose of 10 µl/kg introduced by intravenous bolus injection followed by a 10 ml saline flush, according to the manufacturer's guidelines. More information on the formulation and stability of DEFINITY® microbubbles are included in Appendix 8.1.

4.2 Packaging

New packages of DEFINITY® microbubbles will be shipped from Lantheus Medical Imaging Inc. and will be used in conjunction with the ultrasound transducer to temporarily open the BBB. DEFINITY® microbubbles will be freshly activated before sonication and will be withdrawn using a 1-ml syringe to reach the clinical dose (10 µl/kg) right before the IV injection.

4.3 Potential Risks

All the potential adverse reactions and their incidence rate can be found in the DEFINITY® safety datasheets. There have been reported side effects such as cardiopulmonary reactions, injection site reaction, back/renal pain, chest pain, dizziness, headache, nausea. Other possible risks during the procedure include bruising at the injection site and hypersensitivity or allergic reaction to

DEFINITY® microbubbles and/or gadolinium injections. For further information on DEFINITY® Microbubbles and their adverse events please see appendix 8.1 attached.

5. FOCUSED ULTRASOUND

5.1 Device Description

The system consists of two main parts: (i) ultrasound unit for sonication and real-time passive acoustic monitoring (Sonic Concepts) and (ii) neuro-navigator (Rogue Research) providing real-time guidance. The clinical FUS transducer will be driven by a function generator (33500B Series, Agilent Technologies, Santa Clara, CA, USA) through a 55-dB radiofrequency power amplifier (A150, E&I, Rochester, NY, USA). A water degassing system (WDS105+, Sonic Concepts) will be used to fill the transducer cone with degassed water and inflate or deflate the cone according to the sonicated location. Finally, microbubble acoustic emissions will be recorded (sampling frequency: 50 MHz, capture length: 10 ms) with a 1.5-MHz passive cavitation detector (PCD; diameter: 32 mm, focal depth: 114 mm, ndtXducer, Northborough, MA, USA).
The neuronavigation guidance is achieved by registration of the physical space to the preloaded MRI that is displayed on the screen. The registration is achieved by the infrared camera reflecting the position of the subject tracker secured close to the brain. The transducer with a passive cavitation detector embedded is attached on the mechanical arm and the position varies depending on the brain structure to be sonicated. The transducer carries a tool tracker so its relative position to the brain can be seen on the screen. Once the transducer is locked at the position of interest the sonication begins. Real time monitoring of microbubble activity during sonication will be achieved via passive acoustic detection. This investigative device will be operated in conjunction with DEFINITY® administered intravenously at the FDA-approved dose.

5.2 Device Specification

The FUS device (H-231 FUS transducer, operating band: 0.2 MHz +/- 20% - conversion efficiency: >85% - aperture: 110.0 mm outer diameter x 44.0 mm inner diameter x 110.0 mm radius of curvature, or f/1.0- central Opening: 41.0 mm diameter; Sonic Concepts) consists of a piezoelectric crystal which converts electrical energy into mechanical energy. A function generator is used to produce sinusoidal wave forms which are applied on the transducer through a power amplifier. The RF amplifier depicts the amount of forward and reflected power (Pf and Pr) in real-time. If required, a high-impedance voltage meter will be added at the output channel of the amplifier, to directly measure the output voltage during treatment. The piezoelectric material is surrounded by a matching and backing layer, which allow lossless forward and negligible backward transmission of the ultrasound waves, respectively. The single-element spherical-segment FUS transducer has been designed to have a geometric focus, or radius of curvature, equal to 110 mm. We chose this distance to allow for targeting even in the deepest regions of the human brain. This focal distance was calculated based on numerical simulations of ultrasound wave propagation through the human skull and brain, using the k-wave MATLAB toolbox. A coupling bolus with 5 mm standoff from the exit plane will be filled with degassed water to allow ultrasound propagation into the human brain and reduce heating effects in the skull. A 44-mm central opening was introduced in the FUS transducer to facilitate therapy monitoring via a concentric and co-aligned passive cavitation detector. Microbubble acoustic

emissions will be constantly monitored during therapy. Sonication will be immediately ceased should any unwanted cavitation signals are detected. A custom purpose-built graphics user interface (GUI) will be used to control the emission and reception processes in MATLAB. We have included all the necessary functionalities in this software, enabling full control of the therapeutic process and immediate abortion in the case of an adverse effect. An isolation transformer is indeed included between the main electrical supply and the RF amplifier, which is only high-voltage device of the setup. An emergency stop button has been added in the case of an emergency that would cut off all power from the equipment.

5.3 Device Operation

The investigational device will be operated at acoustic parameters demonstrating safe and effective BBB opening in NHP followed by microbubble injection. The acoustic parameters for BBB opening are based on Dr. Konofagou's NHP studies and correspond to an I_{SPPA} of 2.81 W/cm^2, I_{SPTA} of 56.3 mW/cm^2 and a mechanical index (MI) of 0.4. These parameters produce safe and reproducible BBB opening in NHPs. These values are well below the limits set by the FDA for ultrasound imaging applications (I_{SPPA} < 190 W/cm^2, I_{SPTA} < 720 mW/cm^2 and MI < 1.9, respectively).

The device will be operated at the same specifications as that in adults and other NHP studies since the FUS propagation will be simulated through the skull on a patient-by-patient basis based on the MRI, or CT obtained during radiation planning at the time of diagnosis (if available). CT scans will not be performed for the purposes of this research study. Extensive work has been done at Dr. Konofagou's lab using human skull fragment and simulation, demonstrating that due to the low frequency of the FUS device (i.e., 0.25 MHz), significant skull-induced beam aberrations are not expected. In an adult skull, the focal shift is on the order of a few mm. Therefore, we expect a lower shift through the thinner skull of pediatric patients. The acoustic field will be simulated based on the CT of each patient, if available. Otherwise we will use the MRI scan to acquire structural information about the skull geometry. The 3D simulation will be performed using the kWave toolbox in Matlab. The simulation will be conducted first in free-field (i.e., only water) and then using the CT/MRI scan of the patient, to calculate the attenuation coefficient at a defined beam trajectory and transducer location. No assumptions regarding bone properties are required (if a CT scan is available), since the speed of sound is directly derived from the Hounsfield units of the CT scan. If a CT is not available, we will use the skull contour from the MRI and assume a bone density of 1.35 g/cm^3 (this is the average skull density in pediatric patients [47]). We will perform a total of at least 10 simulations, by allowing a margin of +/- 2mm Euclidian error and +/-20^o angular error. The attenuation coefficient and derated pressure will be calculated as average values across all the simulations. The device will not need maintenance but will be calibrated once every 6 months using a hydrophone. Please see device specifications attached in appendix 7.0.

5.4 Potential Risks

Ultrasound and MRI are generally considered safe imaging modalities. However, there are potential risks related to their usage.

Potential adverse events related to the use of FUS include hemorrhage, and worsening edema. In all human studies, however, no DLTs were observed and there has been no added toxicity due to opening of the BBB through the FUS. Although Zhu et al. [48] has shown that FUS allows evaluation of circulating free DNA raising concerns for tumor dissemination, there has been no preclinical or clinical evidence of increasing the risk for development of metastases in these subjects. We will evaluate and report in the current study whether there has been any rapid development of metastatic disease.

Although ultrasound exposure at high intensities may produce unintended biological effects in the body, such as hemorrhage and edema, our study will be limited to intensities far below the FDA-approved limits for imaging applications (I_{SPPA} < 190 W/cm^2, I_{SPTA} < 720 mW/cm^2 and MI < 0.8). FUS is expected to induce some intended mechanical effects in the brain, but there has been no damage reported in the literature during low-intensity sonication. Sonications at relatively high acoustic pressures have resulted in moderate hemorrhage at the targeted region leading to instances of edema and inflammatory response. We will therefore operate close to the threshold for stimulation to avoid any unwanted effects and minimize the risk for the subject. It is possible that the subject will feel some heat from the ultrasound propagation through the skull. We will account for potential heating by using a cooling medium (i.e., cold water) between the transducer and the skull. All the necessary hardware and software precautions will be taken, in order to eliminate the possibility of transducer malfunction or protocol breach during the clinical sessions.

5.5 Prior and Concomitant Therapy

Although BBB opening is reversible and transient, it may lead to unintended diffusion of pharmaceutical molecules into the brain parenchyma. To avoid such potential effects, medication withholding will be judged based on the subject's diagnosis, condition, and medication type. Examples of medications that will not be withheld include are anti-convulsants steroids and anti-emetics. In all clinical human studies so far despite the use of supportive care and anesthesia no serious adverse events were reported.

5.6 Packaging

The ultrasound transducer and the associated electrical components, i.e. function generator, power amplifier, and hydrophone, will be gathered in a mobile cart. All components will be connected prior to the subject's arrival and will be checked by two investigators to ensure smooth operation and safety.

The neuronavigation system along with the accompanying equipment, i.e. infrared camera, reflecting beads, holders, and other ancillary components, will be packed separately and gathered in a mobile cart. The parts will be assembled following the subject's arrival and the imaging beads will be attached to the subject's head and the transducer to allow for real-time neuronavigation.

5.7 Receiving, Storage, Dispensing and Return

5.7.1 Receipt of Study Device

The device will be delivered to the operating room from the storage room in the investigative site by the researchers prior to the procedure.

5.7.2 Storage

The ultrasound system will be stored in a room equipped with a locker and will be kept under room temperature until usage.

The neuronavigator system will be stored in the same locked room with the ultrasound system, and will also be kept in room temperature until usage.

DEFINITY® microbubble vials will be stored in the Research Pharmacy in a hospital grade refrigerator under 4 ^{o}C until usage. DEFINITY® microbubbles will be removed from refrigerator before the subject's arrival, to allow them to acquire room temperature.

5.7.3 Dispensing of Study Device Agents

The research pharmacy will dispense the DEFINITY® microbubbles and MRI/PET contrast agent. The radiation oncologist on the study will be performing the procedures to the subject, and the other researchers will deliver the ultrasound device from the storage room to the procedure room.

5.7.4 Return or Destruction of Study Device

The device should be returned to the following address if damaged: 630 West 168th Street, Physicians & Surgeons 19-418, New York, NY, USA 10032.

6. STUDY OBJECTIVES

6.1 Primary Objective

The primary objective of this study is to evaluate the feasibility of safely opening the BBB in children with progressive DMG treated with oral Panobinostat using FUS with DEFINITY® microbubbles and neuro-navigator-controlled sonication.

With the current study we are planning to evaluate the safety and feasibility of using FUS and open-space neuronavigator-controlled sonication to open one, two or three tumor sites. For the purpose of the study we will be administrating oral Panobinostat in children with progressive DMG. This drug has a known toxicity profile, dose and well documented efficacy against many metastatic cancers. Successful opening and closing of the BBB will be confirmed with periodic MRIs.

Safety will be assessed by evaluation of physical and neurologic examinations, laboratory studies, radiographic studies, and by adverse events as per the CTCAE version 5.0. An adverse event is

any new or worsening symptom or clinical finding which occurs during the study period. Adverse events are to be recorded irrespective of causality on the adverse event form. Each event will be described by its severity (mild, moderate, severe, life-threatening), duration, and relation to the study medication (unrelated, unlikely, possible, probable, and definite).

6.2 Secondary Objective

- To evaluate the 6-month Progression Free Survival (PFS6) and 6-month Overall Survival (OS6)
- To determine blood brain barrier/tumor imaging changes using FUS in children with progressive DMG

7. STUDY DESIGN

7.1 General Design

The purpose of this study is to evaluate the feasibility of opening the BBB safely using specific parameters of focused ultrasound in progressive/recurrent diffuse midline gliomas in one, two or three tumor sites and receive Oral Panobinostat 15 mg/m^2. The study will consist of a minimum of 3 to a maximum of 15 subjects. See section 7.3 for a treatment schema.

The clinical neuronavigator will be identical to the NHP study with the difference of human module in the software used, as well as employing a separate set of navigation tools that are used for human subjects only.

7.1.1 Subject Recruitment

Subjects of all races and ethnic groups are eligible for this trial.
Subjects will be recruited from the Pediatric Oncology and Pediatric Neurosurgery practices/clinics at Columbia University Irving Medical Center (CUIMC).

7.1.2 Registration Procedures

CUIMC research participant registration

Confirm eligibility as defined in the section entitled Criteria for Subject Eligibility.
Obtain informed consent, by following procedures defined in section entitled Informed Consent Procedures, along with applicable institutional policies and federal regulations.
Only Investigators/Research Personnel properly trained and delegated to consent subjects for this protocol will participate in the consenting process. Furthermore, properly delegated Physician Investigators (e.g., MD, MD-PhD) are required to sign/verify a protocol specific Eligibility Checklist for each subject enrolled on the study, in addition to providing the relevant source documentation confirming subject eligibility.

7.2 Trial Design

The trial will follow a 3+3 “NOTS” or Number of Tumor Sites escalation scheme. The “number of tumor sites” in reference to here in the number of openings in the blood brain barrier using Focused Ultrasound (FUS). Subjects will start the first cycle of the treatment arm with 1 tumor site and move on to incrementing NOTS levels as depicted in Figure 3, if no dose limiting toxicities

(DLTs) are observed (we use the term Dose Limiting Toxicity where *"dose" = Number of Tumor Sites (NOTS)*):
1 tumor site (level "NOTS Level A"),
2 tumor sites (level "NOTS Level B") or
3 tumor sites (level "NOTS Level C")

For a detailed description of DLTs please refer to Section 12.2.

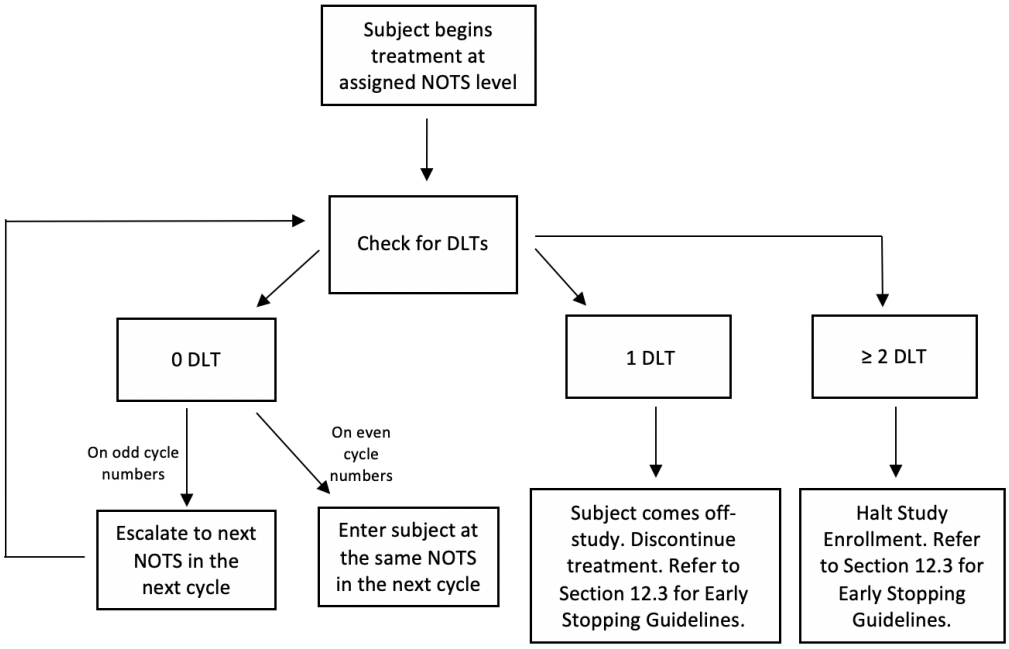


Figure 3: NOTS (Number of tumor Sites) Escalation Schema

For each subject the following FUS NOTS escalation scheme will be followed:

- Each subject will start off with receiving FUS treatment in 1 tumor site (NOTS Level A) for Cycles 1 and 2.
- If 0 DLTs are noted during pre-cycle 3 follow-ups and MRI (without contrast), the subject will receive FUS treatment at 2 tumor sites (NOTS Level B) in the 3rd cycle.
  - If 1 DLT is noted during pre-cycle 3 follow-ups and MRI (without contrast), the subject will discontinue treatments and will come off the study (please refer to section 12.3 for Early Stopping Guidelines). Other subjects may continue to enroll.
  - If 2 DLTs are noted at the same NOTS level, enrollment will be halted following Early Stopping Guidelines detailed in Section 12.3.
- If 0 DLTs are noted during pre-cycle 4 MRI (without contrast) and follow-up, the subject will continue to receive FUS treatment at 2 tumor sites (NOTS Level B) in the 4th cycle.
  - If 1 DLT is noted during pre-cycle 4 follow-ups and MRI (without contrast), the subject will discontinue treatments and will come off the study (please

refer to section 12.3 for Early Stopping Guidelines). Other subjects may continue to enroll.
    - If 2 DLTs are noted at the same NOTS level, enrollment will be halted following Early Stopping Guidelines detailed in Section 12.3.
- If 0 DLTs are noted during pre-cycle 5 MRI (without contrast) and follow-up, the subject will receive the next escalated FUS treatment at 3 tumor sites (NOTS Level C) in the 5th cycle.
    - If 1 DLT is noted during pre-cycle 5 follow-ups and MRI (without contrast), the subject will discontinue treatments and will come off the study (please refer to section 12.3 for Early Stopping Guidelines). Other subjects may continue to enroll.
    - If 2 DLTs are noted at the same NOTS level, enrollment will be halted following Early Stopping Guidelines detailed in Section 12.3.
- If 0 DLTs are noted during pre-cycle 6 MRI (without contrast) and follow-up, the subject will continue to receive FUS treatment at 3 tumor sites (NOTS Level C) in the 6th cycle.
    - If 1 DLT is noted during pre-cycle 6 follow-ups and MRI (without contrast), the subject will discontinue treatments and will come off the study (please refer to section 12.3 for Early Stopping Guidelines). Other subjects may continue to enroll.
    - If 2 DLTs are noted at the same NOTS level, enrollment will be halted following Early Stopping Guidelines detailed in Section 12.3.

Within each cycle, if 1 DLT is noted the subject will discontinue treatments and will come off the study (please refer to section 12.3 for Early Stopping Guidelines). Other subjects may continue to enroll.
If 2 DLTs are noted at the same NOTS level for a subject, study enrollment will be halted following Early Stopping Guidelines detailed in Section 12.3.

7.3 Investigational Plan

Subjects' preoperative MRI and their history will be reviewed at the weekly CUIMC Pediatric Neuro-Oncology Tumor Board to determine disease status, and to confirm eligibility criteria and to schedule the admission date. A recent (no longer than 7 days prior to the time of the procedure) MRI with and without Gadolinium contrast that includes T1, T2, and FLAIR is required, otherwise the MRI will have to be repeated**.**

On the day of admission, the subject will present to the Pediatric Oncology Outpatient clinic and will be brought to the Radiation Oncology Suite (for the Navigator-Guided Focused Ultrasound (NgFUS)) (Children Hospital of New York (CHONY) Floor 3) and subsequently to the Pediatric Oncology outpatient clinic (HIP-7). Institutional policies regarding COVID for transportation will be followed.

In the radiation oncology suite, under the supervision of the Pediatric Oncology and Radiation Oncology teams, the subject will undergo:

- Focused Ultrasound and injection of DEFINITY® Microbubbles using the NgFUS system with the following ultrasonic parameters and microbubble dose:

Table I: NgFUS treatment parameters

FUS frequency	0.25 MHz
Acoustic pressure	200 $kPa_{pk\text{-}neg}$
Pulse length	2,500 cycles or 10 ms
Pulse repetition frequency	2 Hz
Total sonication time per location	2 min
Definity microbubble dose	10 µl/kg
Number of Tumor sites	Level A: 1 Level B: 2 Level C: 3

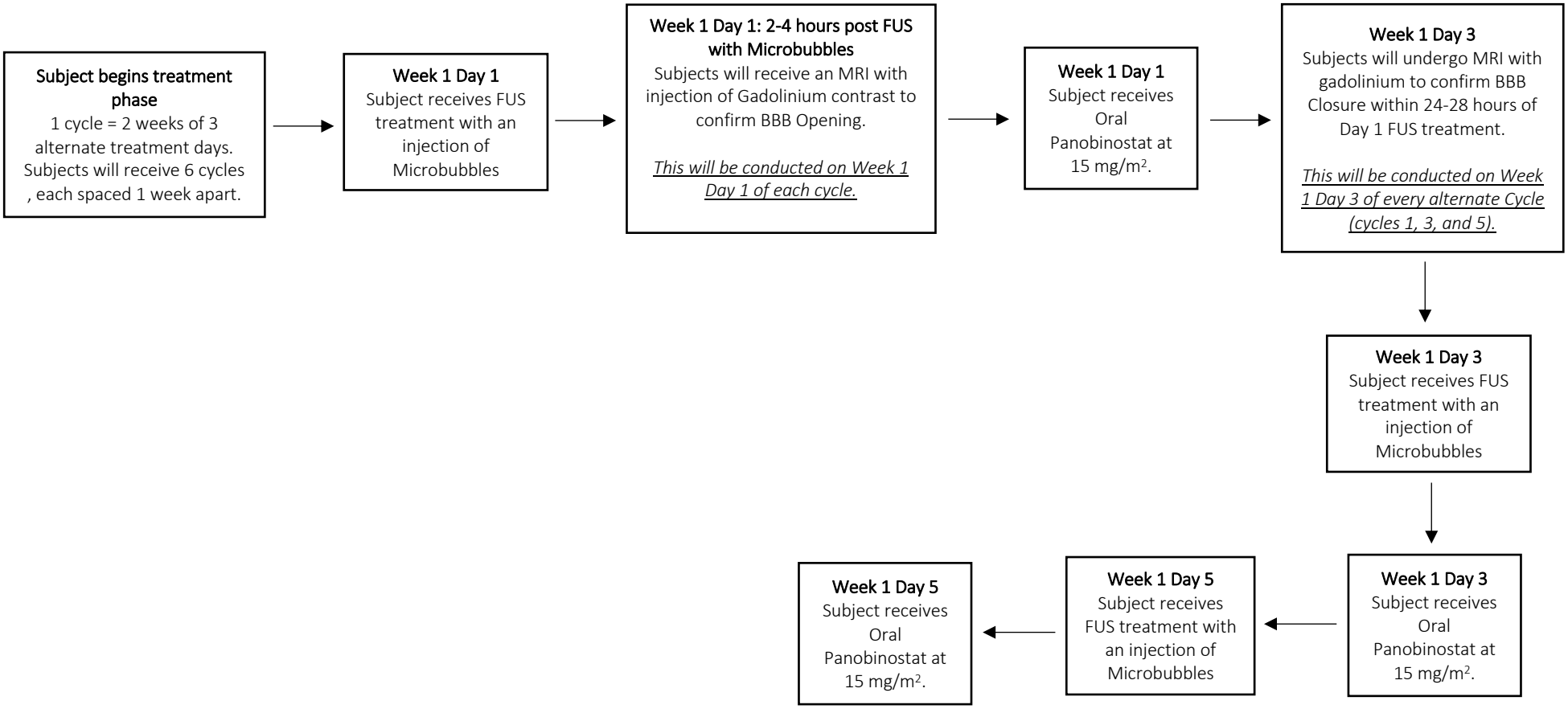
FUS will be aimed for non-enhancing peritumoral areas. Even if there is some contrast enhancement before FUS, this should be clearly increased following FUS. The focal area is quite big (6mm x 6mm x 49mm), so there should be clear opening in the neighboring non-enhancing tissue.

The total estimated time for the FUS procedure is 30-45 min.

Subjects will be sonicated, according to the pre-treatment MRI. Following the NgFUS procedure, subjects will be brought back to the Pediatric Oncology Outpatient clinic where they will receive the oral Panobinostat 15 mg/m^2.

During the mid-cycle at different time-points (as described in section 7.4 and 7.5 below), MRIs with gadolinium contrast will be conducted to ensure proper opening and closure of the BBB. If the subject's MRI shows any anomaly, the subject will be removed from the study and will continue on regular drug treatment as decided by the study team. The subject will follow the schema below.

7.4 Schematic Overview of Panobinostat Treatment for Week 1** Cycle 1



**Week 2 of each cycle will follow a similar schema with the exception of MRIs with contrast conducted to evaluate opening and closing of the BBB.
**Cycles 2 and 4 will follow a similar schema with the exception of an MRI to confirm closure of the BBB on Day 3.

7.5 Treatment MRIs

Treatment MRI breakdown:

- 1 cycle of Panobinostat= 2 weeks of 3 alternate treatment days. Subjects will undergo 6 cycles, each spaced 1 week apart.
- In each cycle, subjects will receive Focused Ultrasound (NgFUS) and injection of DEFINITY® microbubbles using NgFUS system in the Radiation Oncology Suite on Days 1, 3, and 5 (all treatment days).
- **MRI to Determine Opening of Blood Brain Barrier:** On Week 1 Day 1 of each cycle, 2-4 hours post FUS treatment with DEFINITY® microbubbles on Day 1, subjects will receive an MRI with injection of Gadolinium contrast.
- Treatment includes receiving Oral Panobinostat at 15 mg/m^2 on the days of NgFUS treatment.
- **MRI to Confirm Closure of Blood Brain Barrier:** On Week 1 Day 3 of every other cycle (Cycles 1, 3 and 5), subjects will undergo MRI with gadolinium pre Day 3 FUS treatment to ensure closure of the blood brain barrier. This MRI will be conducted within 24-48 hours of the completion of the Day 1 FUS treatment.
- A number of tumor sites (NOTS) escalation scheme for the number of tumor sites targeted by FUS will be followed as laid out in section 7.2. As stated in the section, an MRI (without contrast) will be performed prior to each escalation to clear the subject of any possible DLTs and increase in tumor size.

Subjects will receive FUS treatment in the Radiation Oncology Suite and drug treatments in either the Outpatient Clinic on HIP-7 or in Radiation Oncology immediately after the FUS treatment. Subjects will be monitored by the study team with Neurological Exams on treatment days, as determined necessary. Subjects will not be kept as inpatients for treatment days and will be discharged following completion of treatment day, unless deemed necessary by the study team.

See Appendix A and B on pages 47 and 49 respectively for a detailed breakdown of visits and data collected.

7.6 Opening and Closing of the BBB

7.6.1 Radiological Evidence of the BBB Opening

The BBB will be determined to be open if within 5mm of site of FUS, there is evidence of enhancement seen on T1 weighted post contrast images.

7.6.2 Radiological Evidence of BBB Closure

The BBB will be determined to be closed if within 5mm of site of FUS, there is no evidence of enhancement seen on T1 weighted post contrast images.

7.6.3 Additional MRIs to Confirm BBB Closure

The following steps will be taken if at any time any of the MRIs conducted to confirm whether the BBB is open or closed according to the definitions laid in sections 7.6.1 or 7.6.2 are unmet:

If the MRI to confirm the closure of the BBB on Day 3 does not show evidence of closure:

- subjects will no longer receive another NgFUS treatment on Day 3 to open the blood brain barrier.
- Instead, subjects will receive their scheduled Day 3 dose of Panobinostat and another MRI to confirm closure of the blood brain barrier on Day 5 prior to the scheduled FUS treatment.
- An MRI to confirm closure will be repeated every 48 hours (for a maximum of 3 times) to confirm closure in case of evidence of persistence of opening.
- If the MRI fails to show closure after 3 MRIs, Early Stopping Guidelines and Withdrawal criteria from section 12.3 will be followed.
- Subjects whose BBB takes longer than anticipated to show closure will also receive an additional MRI to confirm closure in the subsequent cycle (even if not planned in the initial study schema).

8. STUDY POPULATION

8.1 Eligibility

8.1.1 Diagnostic Criteria

- Ages 4-21 years.
- Subjects' with evidence of clinical and/or radiographic progression of Diffuse Midline Glioma
- Radiological diagnosis of DMG with tumor involving the pons (intrinsic, pontine based infiltrative lesion; hypointense in T1 weighted images (T1WIs) and hyperintense in T2 sequences, with mass effect on the adjacent structures and occupying at least 50% of the pons), thalami and/or histological confirmation of H3K27M mutation confirmation of pontine or thalamic glioma.

8.1.2 Subject Characteristics

Subjects must be healthy enough to tolerate FUS and MRI and any anesthesia necessary based on the opinion of the principal investigator. Subjects must also be able to swallow capsules (for Panobinostat dosing). Other criteria include, but is not limited to:

Prior therapy:

- Patients must have fully recovered from the acute toxic effects of all prior anti-cancer therapy and must meet the following minimum duration from prior anti-cancer directed therapy prior to enrollment.
    - Cytotoxic chemotherapy or anti-cancer agents known to be myelosuppressive: At least 21 days after the last dose of cytotoxic or myelosuppressive chemotherapy.
    - Anti-cancer agents not known to be myelosuppressive: At least 7 days must have elapsed from last dose of agent.
    - Antibodies: At least 21 days must have elapsed from infusion of last dose of antibody.
    - Interleukins, Interferons, and Cytokines: At least 21 days must have elapsed since the completion of interleukins, interferon, or cytokines.

- Stem cell infusions: At least 42 days must have elapsed after completion of an autologous stem cell infusion, and at least 84 days must have elapsed after completion of an allogeneic stem cell infusion.
- Cellular therapy: At least 42 days must have elapsed since the completion of any type of cellular therapy
- Radiotherapy (XRT): At least 1 month must have elapsed after local XRT.
- Subjects must be on a stable or decreasing dose of steroids, as well as stable dose of anti-seizure medication for 1 week.

Performance status:

- Karnofsky performance status or Lansky play score of ≥70

Hepatic:

- Total bilirubin: within normal institutional limits
- AST(SGOT)/ALT(SGPT): ≤ 2.5 × institutional upper limit of normal

Renal:

- Creatinine: within normal institutional limits
- Creatinine clearance: ≥ 60 mL/min/1.73m^2 for subjects with creatinine levels above institutional normal

Hematopoietic:

- Absolute neutrophil count: ≥ 1,500/µL
- Platelet count: ≥ 100,000/µL
- Hemoglobin level: ≥ 10g/dL
- PT and APTT: within normal institutional limits
- No documented current bleeding disorder

Other:

- Not pregnant or nursing – negative serum pregnancy test, if of childbearing potential, within 7 days of study entry
- Subjects with a history of seizures/epilepsy should be on anti-convulsant medication prior to the first operative procedure on study.
- Subjects must undergo a baseline EKG within 7 days of study enrollment.
- Subjects must be able to undergo MR imaging with gadolinium-based contrast administration (e.g. no ferrous-containing implants, no pacemakers, etc.)
- All subjects or their legal guardians must sign a document of informed consent indicating their understanding of the investigational nature and the potential risks associated with this study. When appropriate, pediatric subjects will be included in all discussions in order to obtain verbal and written assent

8.1.3 Exclusion Criteria

- Subjects with spinal DMGs.
- Subjects with a medical condition that would preclude general anesthesia
- Subjects with evidence of any active infection
- Subjects with documented allergy to compounds of similar chemical or biologic composition to Panobinostat or gadolinium compounds
- Subjects with evidence of tumor hemorrhage

- Subjects with an uncorrectable bleeding disorder
- Subjects with signs of impending herniation or an acute intratumoral hemorrhage
- Subjects with systemic diseases which may be associated with unacceptable anesthetic/operative risk
- Subjects with implanted electrical devices, metallic implants
- Subjects with uncontrollable hypertension
- Subjects with a history of stroke or cardiovascular disease
- Subjects with cerebrovascular diseases
- Subjects with coagulopathy or under anticoagulant therapy.
- Pregnant or breast-feeding women will not be entered on this study, since there is yet no available information regarding human fetal or teratogenic toxicities. A pregnancy test must be obtained in girls who are post-menarchal. Males with female partners of reproductive potential or females of reproductive potential may not participate unless they have agreed to use two effective methods of birth control- including a medically accepted barrier method of contraception (e.g., a male or female condom) for the entire period in which they are receiving protocol therapy and for at least 1 week following their last study treatment requirement. Abstinence is an acceptable method of birth control.

9. FOLLOW-UP

9.1 Duration of Therapy and Follow-up

The treatment period of this study begins at the time of the NgFUS ultrasound and concludes upon evidence of progression . Patients will be monitored for AEs and SAEs for up to 90 days post completion of study. During follow-up the study team will be responsible for conducting a physical exam, repeating MRI (if indicated by the primary physician), and collecting survival data.

10. MEASUREMENT OF EFFECT

The MRIs should include volumetric sag T1 pre- and post- contrast images with reconstructions as well as dynamic contrast enhanced images (which will be acquired over a 30 minute period to allow for sufficient time for the contrast to cross the blood brain barrier site which was opened with FUS), axial post contrast SE T1 as well as sag T2 volumetric (with coronal and axial reconstructions) (can be acquired post-contrast), axial T2 FSE (can be acquired post-contrast), axial SWAN, axial FLAIR (must be acquired pre-contrast), and axial DWI (must be acquired pre-contrast). Initially, we will ideally choose the area for opening of the blood brain barrier with NgFUS at a peripheral site of the tumor, which does not demonstrate post contrast enhancement on the pre-treatment MRI. We will choose one site for blood brain barrier opening. We can measure contrast enhancement as described in Mainprize et al as well as has previously been measured by members of our group in past work (by investigators Pouliopoulos and Konofagou). Radiological evidence of successful BBB opening and closing will also be noted as mentioned in section 7.6.

10.1 Sample Size Determination

A minimum of 3 and maximum of 15 patients will be recruited in the study. The minimum number of subjects is to account for “dose” limiting toxicities disallowing more subjects to be enrolled. As this is a feasibility study, the power and sample size is not justified. However, successful methodology will be concluded if the BBB opening is safely opened in at least 75% of the cases as evidenced on MRI. The scan with the BBB opening will be compared to the scan before BBB opening for each patient. Power analysis shows that for a detectable difference of 72 mm^3 (after BBB opening - before BBB opening) and a standard deviation of 50 mm^3, at least 6 subjects are needed to report a result at the level of α=0.05 and Power=0.80 with paired student’s t-test.

10.2 Statistical Data Analysis

The data analysis will be descriptive. The continuous scan measures will be summarized by median, minimum and maximum. The categorical measures will be summarized by frequency and proportions, such as adverse events. Nonparametric Wilcoxon signed-rank test will be used to compare the gadolinium enhancement in the patient’s brain before and after BBB opening.

11. ADVERSE EVENT REPORTING

11.1 Definitions

Adverse Event

An adverse event (AE) is any untoward or unfavorable medical occurrence in a human subject, including abnormal sign, symptom or disease associated with the subject's participation in research, whether or not considered related to the subject's participation in the research. For the treatment period and 30 days post, all adverse events (AEs) according to the Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 will be captured. Baseline abnormalities or pre-existing conditions will be identified and recorded as part of the pre-treatment work-up. Pre-existing conditions will not be subsequently reported as adverse events unless the severity increases after the start of treatment, or unless the pre-existing condition resolves and then recurs during treatment. After day 30 of follow-up, only grades 3, 4, and 5 AEs will be captured through 90 days following the neurosurgical procedure. All recorded AEs must have their attribution to the study procedures recorded, as well as their onset and resolution date (if known).

Serious Adverse Event

A serious adverse event (SAE) is any AE that is:

- fatal
- life-threatening
- requiring inpatient hospitalization/prolongation of existing hospitalization, unless:
    - routine treatment or monitoring of the studied indication, not associated with any deterioration in condition (procedures such as central line placements, paracentesis, pain control)
    - elective or pre-planned treatment for a pre-existing condition that is unrelated to the indication under study and has not worsened since the start of study the drug
    - social reasons and respite care in the absence of any deterioration in the subject's general condition
- resulting in persistent or significant disability or incapacity
- a congenital anomaly or birth defect
- an important medical event

Important medical events are those that may not be immediately life threatening but are clearly of major clinical significance. They may jeopardize the subject and may require intervention to prevent one of the other serious outcomes noted above.

Any adverse event that results in hospitalization or prolonged hospitalization should be documented and reported as a serious adverse event unless the AE meets one of the criteria listed below:

- Hospitalization or prolonged hospitalization for diagnostic or elective surgical procedures for a preexisting condition. Surgery should not be reported as an outcome of an adverse event if the purpose of the surgery was elective or diagnostic and the outcome was uneventful

- Hospitalization or prolonged hospitalization required to allow efficacy measurement for the study
- Hospitalization or prolonged hospitalization for therapy of the target disease of the study, unless it is a worsening or there is increase in the frequency of hospital admissions as judged by the clinical investigator
- Prolonged stay at MSCHONY at the Investigator's discretion unless it is for an adverse event that otherwise qualifies as an SAE

Unanticipated Problem

An unanticipated problem is any incident, experience or outcome involving risks to subjects or others in any human subject research that meets all of the following criteria:

- Unexpected (in terms of nature, severity or frequency) given (a) the research procedures that are described in the IRB-approval protocol and informed consent document, and (b) the characteristics of the subject population being studied;
- Related or possibly related to participation in such research (e.g., there is a reasonable possibility that the incident, experience or outcome may have been caused by the procedures involved in such research); and
- Suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic or social harm) than was previously known or recognized.

All unanticipated problems meeting the criteria above will be reported to the IRB within 1 week of investigator/study staff awareness of the event (or other time frame required by the most current IRB reporting policies). Additionally, the sponsor-investigator will submit a summary of all unanticipated problems that occurred since the beginning of the study at the time of continuing review. Copies of each report and documentation of IRB notification and receipt will be kept in the Regulatory Binder.

Suspected Adverse Reaction (SAR)

In accordance with 21 CFR 312.32(a), a suspected adverse reaction (SAR) means any adverse event for which there is a reasonable possibility that the drug caused the adverse event.

Unanticipated Adverse Device Effect (UADE)

An unanticipated adverse device effect (UADE) is any serious adverse effect on health or safety or any life-threatening problem or death caused by, or associated with, a device, if that effect, problem, or death was not previously identified in nature, severity, or degree of incidence in the investigational plan or IDE application (including a supplementary plan or application), or any other unanticipated serious problem associated with a device that relates to the rights, safety, or welfare of subjects.

Associated with the investigational device: There is a reasonable possibility that the adverse effect may have been caused by the investigational device.

11.2 Recording of Suspected Adverse Reactions (SARs) and Adverse Device Effects (ADEs)

All observed or volunteered adverse effects (serious or non-serious) and abnormal test findings, regardless of treatment group, if applicable, or suspected causal relationship to the investigational products or, if applicable, other study treatment or diagnostic product(s) will be recorded in the subjects' case histories. For all adverse effects, sufficient information will be pursued and/or obtained so as to permit 1) an adequate determination of the outcome of the effect (i.e., whether the effect should be classified as a serious adverse effect) and; 2) an assessment of the causal relationship between the adverse effect and the investigational products or, if applicable, the other study treatment or diagnostic product(s).

Adverse effects or abnormal test findings felt to be associated with the investigational device or, if applicable, other study treatment or diagnostic product(s) will be followed until the effect (or its sequelae) or the abnormal test finding resolves or stabilizes at a level acceptable to the sponsor-investigator.

11.3 Reporting of Suspected Adverse Reactions, Adverse Device Effects and Unanticipated Problems

11.3.1 Investigator Reporting: *Notifying the study sponsor*

N/A

11.3.2 Investigator Reporting: *Notifying the IRB*

ALL unanticipated events that meet the definition of an Unanticipated Problem will be reported within 1 week of becoming aware of the event. Expected or unrelated adverse effects and potential protocol violations will be recorded and reported in the annual IRB renewal submission. Additionally, the sponsor-investigator will submit a summary of all unanticipated problems that occurred since the beginning of the study at the time of continuing review. Unanticipated events that are not considered serious adverse events, will be reported at the time of continuing review. Copies of each report and documentation of IRB notification and receipt will be kept in the Regulatory Binder.

11.3.3 Investigator Reporting: *Notifying the FDA*

The Sponsor-Investigator (S-I), as holder of the IND, will be responsible for all communication with the FDA and will report to the FDA, any adverse event that is serious, unexpected and for which there is reasonable possibility that the drug caused the adverse event. These must be reported to the FDA as soon as possible, but no later than 15 calendar days after the S-I determines that the information qualifies for reporting. Any fatal or life-threatening SARs will be reported to the FDA as soon as possible, but no later than 7 calendar days after the S-I determines that the information qualifies for such reporting.

The Columbia University Irving Medical Center Sponsor-Investigator must report to the FDA and any affiliate site investigators as follows:

- Any unexpected fatal or life-threatening event must be reported as soon as possible, but no later than 7 calendar days after the sponsor-investigator's initial receipt of the information
- Any findings from epidemiological studies, pooled analysis of multiple studies, or clinical studies, whether or not conducted under an IND, and whether or not conducted by the sponsor-investigator, that suggest a significant risk in humans exposed to the drug must be reported as soon as possible but no later than 15 calendar days after the sponsor-investigator determines that the information qualifies for reporting
- Any findings from animal or in vitro testing whether or not conducted under an IND, and whether or not conducted by the sponsor-investigator, that suggest a significant risk in humans exposed to the drug must be reported as soon as possible but no later than 15 calendar days after the sponsor-investigator determines that the information qualifies for reporting
- Any clinically important increase in the rate of a serious suspected adverse reactions over that listed in the protocol or Investigator Brochure
- Expected SAEs and AEs will be included in the IND Annual Reports

The Sponsor-Investigator will report any UADE that meet the reporting criteria to the FDA within 10 days of the investigator/team becoming aware of the event.

Reportable Adverse events may be submitted on FDA Form 3500A, a Serious Adverse Event Reporting Form, or in a narrative format.

Updates and follow up reports will be submitted as soon as the updated information becomes available to the investigator and/or research team.

11.3.4 DSMC Reporting by sponsor-investigator

Serious adverse events not constituting unanticipated problems are to be reported to the HICCC DSMC. Reporting should occur within 24 hours of knowledge of the SAE occurring at our institution or affiliate sites.

11.3.5 Reporting to Drug Manufacturer by sponsor-investigator

The Sponsor-Investigator will report any serious adverse events that meet the reporting criteria to the Institutional Review Board and DSMC as described above. Since drug stock is being purchased directly from commercial entities, there is no need for adverse event reporting directly to the drug manufacturer.

11.3.6 Reporting Process

Reportable adverse events may be submitted on FDA Form 3500A, the HICCC DSMC Serious Adverse Event Reporting Form, or in a narrative format.

12. DOSE MODIFICATION AND WITHDRAWAL CRITERIA

12.1 Dose modification

Doses of the drug (Panobinostat) will not be reduced. Instead, if the patient recovers from any previous adverse event (AE) or Serious Adverse Event (SAE) and the principal investigator determines that it is in the patient's best interest to proceed with the study therapy for maximal therapeutic benefit, the next scheduled FUS/treatment should be administered. During any cycle, if grade 2 or higher toxicity is unresolved before the next scheduled FUS treatment, treatment will be halted and will be re-evaluated upon resolution of toxicity. The treatment may be resumed for the subject if the toxicity is resolving before the subsequent scheduled FUS treatment.
If the treatment related toxicity experienced toxicity (Grade 2 or higher) persists for more than 21 days or there is ≥1 Dose Limiting Toxicity (DLT), the subject will not have the subsequent FUS.
If two DLTs occur at the same NOTS level, further enrollment will be halted.
Additional modifications to timing of doses may be performed at the discretion of the PI.

General guidelines: Patients with minor signs of increased intracranial pressure may be managed by steroids and mannitol. If patients develop significant new neurologic deficits or a change in level of consciousness (Grade 2 or higher symptomatic intracranial hemorrhage or stroke), the treatment will be stopped.

Criteria to proceed to subsequent FUS/treatment include :

- Absence of intercurrent active infection or intercurrent illness
- Resolving treatment related toxicity experienced in prior FUS /treatment to grade 2 or less
- Patient decides to withdraw

Within each cycle, if 1 DLT (defined in Section 12.2) is noted the subject will discontinue treatments and will come off the study (please refer to section 12.3 for Early Stopping Guidelines). Other subjects may continue to enroll.
If 2 DLTs are noted at the same NOTS (Number of Tumor Sites) level for a subject, study enrollment will be halted following Early Stopping Guidelines detailed in Section 12.3.

Subjects as well as their family members will be educated about signs and symptoms of increased intracranial pressure and neurological deficit, for which the study investigators should be notified, and emergent medical attention should be sought.

This protocol will be uniform for all subjects enrolled in the trial.

12.2 Dose Limiting Toxicities

Dose limiting toxicities (DLT) will be defined as any new (or increased from baseline), treatment related (drug and/or device):

1. Neurological deficits based on neurological examination which are grade 3 or greater nervous system adverse events at least possibly attributed to sonication (as defined by CTCAE v5.0).

2. Grade 2 or higher symptomatic intracranial hemorrhage or stroke.

These deficits include but are not limited to changes in level of consciousness, new onset speech difficulties (aphasia, mutism, dysarthria), confusion, visual field deficits, focal weakness, seizures. The DLT period is defined as starting the day of the first scheduled FUS treatment and lasting until 14 days after the last scheduled FUS treatment.

Furthermore, DLT criteria include any systemic treatment- related Grade 3 or higher hematologic or non-hematologic toxicity within a period of 14 days after the scheduled FUS treatment. Examples of exceptions could include:

I. Grade 3 thrombocytopenia without bleeding, Grade 3 or 4 lymphopenia, Grade 3 neutropenia without fever.
II. Nausea, vomiting or diarrhea responding to medical management within 48 hours
III. Electrolyte disturbances that are asymptomatic and response to supportive care within 48 hours; hyperglycemia ≤12 hours

12.3 Early Stopping Guidelines

In the event 2 DLTs occur for more than 1 subject at a NOTS (number of tumor sites) level (as defined in section 7.2), study enrollment will be immediately halted. There will be at least 14 days following completion of the FUS treatment of the patient(s) on study before any subsequent patient is enrolled. After re-evaluation and consultation with the HICCC DSMC board, the trial may re-initiate enrollment with adjusted treatment parameters after a consensus is agreed upon by the Sponsor-Investigator and the HICCC DSMC, and a subsequent modification is reviewed/approved by the local PRMC/IRB. If a consensus cannot be reached, no further enrollment will be permitted on the trial and closure procedures will be initiated. In addition, the study will be discontinued at any given time if more than 20% of patients experiencing medical complications including the development of significant, irreversible deterioration during the treatment. With this rule and under the assumption of the true probability of having the medical complication is 10%, the probability of stopping the study due to the medical complication is 0.02 when the targeted total sample size is 6 and is 0.053 when the targeted total sample size is 9.

If the MRI to confirm opening of the BBB for any subject is irregular at any time during the cycles, the subject will not receive the scheduled dose treatment until a decision is made by the investigating team.

If the MRI confirm the closure of the BBB for any subject are irregular at any time, the subject will not receive any further FUS treatments and will receive another MRI to confirm closure every 48 hours for a maximum of 3 times. If the subject does not show BBB closure after 3 MRIs, the subject will come off the study. The subject will be able to receive the scheduled dose of drug, unless otherwise specified by the investigating team.

Response and progression will be evaluated in this study using the international criteria proposed by the updated Pediatric Response Assessment in Neuro-oncology (RANO) guidelines[49].

Criterion	Complete Response	Partial Response	Stable Disease	Progressive Disease
TI-weighted post-Gd measurable disease	None	≥50% decrease	<50% decrease but <25% increase	≥25% increase
T2/FLAIR	Stable or decreased	Stable or decreased	Stable or decreased	Increased
New lesion	None	None	None	Present
Corticosteroids	None	Stable or decreased	Stable or decreased	NA
Clinical status	Stable or increased	Stable or increased	Stable or increased	Decreased
Requirement for response	All	All	All	Any

12.4 Early Withdrawal of Subjects

- If the subject fails to comply with the defined treatment plan and follow-up evaluations, the subject may be removed from the study at the discretion of the treatment investigator
- If the subject withdraws consent for continued participation, he/she will be removed from the study
- If the subject develops significant, new neurologic deficits or a change in level of consciousness a CT or MR scan may be performed to evaluate for hemorrhage/edema. If symptoms remain unmanageable by medications, craniotomy may be performed for resection of tumor, necrosis, or hemorrhage if medically indicated
- The reason for study removal and the date the subject was removed should be documented in the Case Report Form (CRF)
- If the treatment related toxicity experienced toxicity (Grade 2 or higher) persists for more than 21 days or ≥1 Dose Limiting Toxicity, the subject will not have the subsequent FUS and early withdrawal will be considered.

The primary endpoint of this study is safety. As such, subjects will be withdrawn from the study as per the guidelines outlined above. Should similar toxicities occur in more than one subject; early interruption of the study will be considered.

12.5 Data collection and follow-up for withdrawn subjects

Even though subjects may be withdrawn prematurely from the study, it is imperative to collect at least survival data on such subjects throughout the protocol, defined follow-up period (though careful thought should be given to the full data set, which should be collected on such subjects to fully support the analysis). Such data is important to the integrity of the final study analysis since early withdrawal could be related to the safety profile of the study drug. If a subject withdraws consent to participate in the study, attempts will be made to obtain consent from the subject to record at least survival data up to the protocol-described end of the subject follow-up period. It must be a high priority to try to obtain at least survival data on all subjects lost to follow-up and to note what methods should be used before one can state the subject is truly lost to follow-up (e.g. number of phone calls to subject, phone calls to next-of-kin if possible, certified letters, etc.). Subjects who have received study drug and are withdrawn because of unacceptable adverse events will be followed until resolution or stabilization of the adverse event.

12.6 Stopping Rules

Redundant safety features have been added to the hardware of the device, as grounding for the transducer, safety switch for the amplifier and function generator. Additionally, we will incorporate safety valves in our software. For any signs showing safety risk including high cavitation dose in the acoustic monitoring or unstable vital signs, the device can be stopped from the computer user interface and/or an emergency stop button. Treatment will be stopped in the case of any reaction of the subject to the ultrasound exposure or microbubble infusion. Pulse oximetry will be used during MRI scans to mitigate the risk of anaphylactic reaction to MRI contrast agents.

The investigation will be terminated in case of any death, life threatening event, or any other serious adverse event (including disability or clinically significant manifestation of local hemorrhage or edema) due to the procedure. An episode of status epilepticus during treatment will terminate the session immediately.

If the selected ultrasound settings (i.e., MI of 0.4) do not successfully open the BBB in 3 subjects, FDA will be contacted via a submission of an IND supplement, to discuss revising the protocol's focused ultrasound parameters.

13. DATA REPORTING/REGULATORY REQUIREMENTS

13.1 Data Reporting

Case Report Forms will be completed for each subject enrolled into the clinical study through an electronic data capture system called Velos. It is the investigator's responsibility for ensuring that all clinical and laboratory data entered on the corresponding CRFs are complete, accurate and authentic.

13.2 Medical Monitoring

Medical monitoring will be performed by either the PI, Dr. Cheng-Chia Wu, lead neuro-oncologist of the trial, Dr. Luca Szalontay, the neuro-oncology fellow, Dr. Andrea Webster-Carrion, the research nurse, Jessica Morcone, or any other trained oncologist and/or radiation oncologist present during the trial. He/She will assess the subject's clinical status during and after the therapy/procedure. In the case of an allergic or any other reaction (e.g. status epilepticus) to the therapy, the subject will be withdrawn. Resuscitation equipment will be readily available in the study room, in case of a serious adverse event.

Subjects will be monitored with follow-up MRI scans to confirm BBB opening and closing. Should the MRI of this study have incidental findings (e.g. brain tumor, subarachnoid hemorrhage, or structural abnormality) the subject will be immediately withdrawn from the study and directed for clinical care. MRI scans will be examined by a qualified physician who is a certified in Diagnostic Radiology. MRI scan examination by a qualified physician will occur as soon as

possible but no later than two weeks following receipt of the image.

Incidental findings will be communicated with both the subject and the subject's personal primary care physician. Communication with the subject will be the responsibility of the Sponsor/Investigator of this study, Dr. Cheng-Chia Wu. Communication may be initially verbal, followed by a written communication, and will include a classification of the incidental finding, i.e. class A – life-threatening/severe – or class B – important but not severe. Incidental findings of clinical significance and the management of such findings should be documented in the research records of this study.

At the time of a continuing review, if incidental findings were noted during the previous approval period, the principal investigator of this study will provide the IRB with the following information: the number of required review images, a list of the subject study numbers, the type of scan, the date of the scan, a description of the incidental finding of clinical significance, the date of communication with the subject and the outcome, if known.

13.3 Data and Safety Monitoring Committee

To ensure the long-term safety in our study, the investigators will monitor and collect the data for processing and analysis while Dr. Zacharoulis, or another co-investigator physician will be present to assess the safety of the patient during NgFUS application. Dr. Zacharoulis will oversee the study procedures and will assess the subject's clinical status during therapy. In the case of an allergic or any other reaction to the therapy, the study will be immediately discontinued. Resuscitation equipment will be readily available in the study room, in case of a serious adverse event.

The NCI-approved Data Safety and Monitoring Committee (DSMC) of the Herbert Irving Comprehensive Cancer Center (HICCC) will monitor every subject who receives treatment on this protocol for toxicity. This protocol will adhere to the policies of the currently approved HICCC Data and Safety Monitoring Plan (DSMP), which is in accordance with NCI and CUMC-IRB policy and guidelines. The committee chair is appointed by the HICCC Director. The committee consists of HICCC faculty and staff with expertise in oncology, research pharmacy, research nursing, and data management. The DSMC convenes twice a month to review patient safety and the conduct of the trial. The PI will submit data and safety monitoring reports to the DSMC at a frequency to be determined by the DSMC based on risk to the subjects.

At the time of renewal, the study team will submit the most recent DSMC approval letter for safety review to the CUMC IRB. Any modifications that are required by the DSMC to ensure patient safety will be submitted to the IRB. All protocol deviations, violations, and eligibility waivers will be submitted to and approved by the DSMC prior to being reported to the IRB. All study data reviewed and discussed during these meetings will be kept confidential.

13.4 Quality Control and Quality Assurance

Independent monitoring of the clinical study for protocol and GCP compliance will be conducted periodically by the the departmental QA. Additionally, the Compliance Oversight Committee of the IRB at Columbia University Irving Medical Center may audit the study at any time per institutional policies and procedures. The investigator-sponsor and Columbia University Irving Medical Center will permit direct access of the study monitors and appropriate regulatory authorities to the study data and to the corresponding source data and documents to verify the accuracy of this data.

A risk-based approach will be used by the departmental QA to determine the frequency, number of subject charts, and data elements to be monitored. The departmental QA will review the study status and summarize enrollment, toxicities, SAEs/UPs, dose escalation, statistical endpoints (e.g., stopping rules), etc. for the full DSMC membership at the regularly scheduled meetings.

13.5 Internal On-Site Monitoring

The study Monitoring Visit Log will be completed and signed by the monitor and the PI/CRNP/CRN and/or CRC and will be filed in the regulatory binder.

The departmental QA will communicate with the site coordinator/site Principal Investigator to schedule the monitoring visit and arrange for access to study materials and documentation.

The assigned departmental QA will monitor investigator-initiated trials within 1 month after the first subject is enrolled and throughout the life of the study to ensure that the study is being conducted in accordance with the protocol, GCP, applicable federal and local regulations, and per all applicable SOPs. The departmental QA is responsible to notify the PI and CRNP/CRN/CRC of upcoming monitor visits and convey what information and documentation will be required for the visit(s). The departmental QA is responsible for verifying that informed consent is properly obtained, eligibility is met (via the central registration process), and all study procedures are conducted according to the study protocol. The departmental QA will also verify that the data reported in the CRF's accurately reflect source documents, that all toxicities have been reported to date, and that all SAE's/UPs/deviations/violations have been reported according to local IRB and HICCC DSMC requirements. The departmental QA will issue queries and ensure resolution in a timely and efficient manner. The departmental QA will also monitor for applicable regulatory compliance and research pharmacy compliance (if applicable) and communicate any deficiencies as appropriate.

13.6 Confidentiality

Information about study subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act (HIPAA). Those regulations require a signed subject authorization informing the subject of the following:

- What protected health information (PHI) will be collected from subjects in this study
- Who will have access to that information and why
- Who will use or disclose that information

- The rights of a research subject to revoke their authorization for use of their PHI
- In the event that a subject revokes authorization to collect or use PHI, the investigator, by regulation, retains the ability to use all information collected prior to the revocation of subject authorization. For subjects that have revoked authorization to collect or use PHI, attempts should be made to obtain permission to collect at least vital status (e.g., that the subject is alive) at the end of their scheduled study period.

The subject binders (physical and electronic) will be maintained within the Clinical Research Coordinator (CRC) and Principal Investigator (PI) offices, a secured floor within the Herbert Irving Pavilion and only the investigator and study staff will have access to the file.

13.7 Source documents

Source data is all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents. Examples of these original documents, and data records include: hospital records, clinical and office charts, laboratory notes, memoranda, subjects' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, subject files, and records kept at the pharmacy, at the laboratories, and at medico-technical departments involved in the clinical trial.

13.8 Case report forms

The study case report form (CRF) is the primary data collection instrument for the study. All data requested on the CRF will be recorded. All missing data will be explained. If a space on the CRF is left blank because the procedure was not done or the question was not asked, "N/D" will be documented. If the item is not applicable to the individual case, "N/A" will be documented.

13.9 Record retention

Records relating to a specific research activity, including research records collected by investigators, will be maintained for the minimum number of years as outlined in all applicable regulations (45 CFR 46.115(b); 21 CFR 56.115(b); 21 CFR 812.140(d)). This minimum retention period will apply whether or not any subjects were enrolled in the study.

Clinical records, including consent forms that document clinical intervention, clinical diagnostic procedure, or research-related procedure, will be retained in medical records by the institution for at least seven years per CUMC and NYP policy, which is based on state law.

14. PROTECTION OF HUMAN RIGHTS

This study is to be conducted in accordance with applicable government regulations and institutional research policies and procedures.

This protocol, and any amendments, will be submitted to a properly constituted Institutional Review Board (IRB), in agreement with local legal prescriptions, for formal approval of the study conduct. The decision of the IRB concerning the conduct of the study will be made in writing to the investigator and a copy of this decision will be obtained before commencement of this study. All subjects for this study will be provided a consent form (and an assent for minors) describing this study and providing sufficient information for subjects to make an informed decision about their participation in this study. This consent form will be submitted with the protocol for review and approval by the IRB for the study. The formal consent of a subject, using the IRB-approved consent form, must be obtained before that subject is submitted to any study procedure. The subject must sign this consent form or legally acceptable surrogate, as outlined in the IRB approved protocol, and the investigator-designated research professional obtaining the consent.

15. PUBLICATION PLAN

Neither the complete nor any part of the results of the study carried out under this protocol, nor any of the information provided by the sponsor for the purposes of performing the study, will be published or passed on to any third party without the consent of the study sponsor. Any investigator involved with this study is obligated to provide the sponsor with complete test results and all data derived from the study.

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Appendix A- Sequence of Events

An enumerated list of chronological study procedures has been provided below. Please see Appendix B for a table version.

Pre-study (up to 7 days before the NgFUS procedure):

1. Patient Recruited
2. Enrollment Criteria Verified
3. Informed Consent Signed
4. MRI with and without IV Gadolinium contrast (A recent (no longer than 7 days prior to the time of the procedure) MRI with and without Gadolinium contrast that includes T1, T2, and FLAIR is required, otherwise the MRI will have to be repeated).
5. Pre-Study Information Collected
    a. Medical History
    b. Physical Exam
    c. Vital Signs
    d. Height
    e. Weight
    f. Karnofsky/Lansky Performance Status
    g. Neurocognitive Assessment Performed
    h. Basic labs/pre-op Testing Collected and Reviewed
        i. CBC with differential
        ii. Serum Chemistry (BMP and hepatic function panel)
        iii. Coagulation Panel (PT, INR, APTT)
6. Subject starts treatment.

NgFUS Procedure:

Subjects will be assigned a NOTS (number of tumor sites) level pertaining to the number of tumor sites FUS treatment will be applied to as laid out in Section 7.2.

Panobinostat Treatment Schedule:

- 1 cycle of Panobinostat= 2 weeks of 3 alternate treatment days. Subjects will undergo 6 cycles spaced 1 week apart.
- In each cycle, subjects will receive Focused Ultrasound (NgFUS) and injection of DEFINITY® microbubbles using NgFUS system in the Radiation Oncology Suite on Days 1, 3, and 5 (all treatment days).
- **MRI to Determine Opening of Blood Brain Barrier:** During Week 1 Day 1 of each cycle, 2-4 hours post FUS treatment with DEFINITY® microbubbles on Day 1, subjects will receive an MRI with injection of Gadolinium contrast.

Post NgFUS Procedure (+4-24 hours):

Panobinostat Treatment Schedule:

- Treatment includes receiving Oral Panobinostat at 15 mg/m^2.
- **MRI to Confirm Closure of Blood Brain Barrier:** On Week 1 Day 3 of every other cycle (Cycles 1, 3 and 5), 24-48 hours post Day 1 FUS treatment with DEFINITY® microbubbles, subjects will undergo MRI with gadolinium pre Day 3 FUS treatment to ensure closure of the blood brain barrier.
- A NOTS escalation scheme for the number of tumor sites targeted by FUS will be followed as laid out in section 7.2. As stated in the section, an MRI (without contrast) will be performed prior to each escalation to clear the subject of any possible DLTs and increase in tumor size.

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Follow-up Period (until 90 days after treatment):

1. Patients will be monitored for AEs and SAEs for 90 days post-treatment.
2. An MRI (with Gadolinium contrast) will be done in the follow-up period as determined by the study doctor.

Appendix B- Study Calendar

- 1 cycle of Panobinostat= 2 weeks of 3 alternate treatment days. Subjects will undergo 6 cycles, each spaced 1 week apart.
- In each cycle, subjects will receive Focused Ultrasound (NgFUS) and injection of DEFINITY® microbubbles and using NgFUS system in the Radiation Oncology Suite on Days 1, 3, and 5 (all treatment days).

	Screening	**Treatment Phase**				**Follow-up**
Time points	**-7 days**	**Day 1**	**+12-+48hours**	**Day +3**	**Day +5**	**+90 days**
Height	X					X
Weight	X					X
Medical History	X					
Demographics	X					
Vital signs	X					X
Informed Consent	X					
CBC w/diff, plts	X		X[c]			X
Serum chemistry (BMP and hepatic function panel)	X		X[d]			X
Coagulation Panel (PT, INR, PTT)	X		X[e]			
Concomitant Medication Review	X					X
EKG	X		X[f]			
Focused Ultrasound Treatment		X[b]		X[b]	X[b]	
Performance Status	X	X				X
MRI	X[a]		X[g]	X[i]		X[j]
Oral Panobinostat		X[h]		X[h]	X[h]	
Physical Exam			X[k]			

a- MRI with gadolinium contrast to be performed for screening purposes.
b- In each cycle, subjects will receive Focused Ultrasound (NgFUS) and injection of Microbubbles using NgFUS system in the Radiation Oncology Suite on Days 1, 3, and 5 (all treatment days).
c- Perform one approximately 12-24 hours post NgFUS
d- Perform once approximately 12-24 hours post NgFUS
e- Perform once approximately 12-24 hours post NgFUS.
f- An electrocardiogram (EKG) will be performed once approximately 12—48 hours post NgFUS.
g- **MRI to Determine Opening of Blood Brain Barrier:** During Week 1 Day 1 of each cycle, 2-4 hours post FUS treatment with Microbubbles on Day 1, subjects will receive an MRI with injection of Gadolinium contrast.
h- Treatment includes receiving Oral Panobinostat at 15 mg/m^2.
i- **MRI to Confirm Closure of Blood Brain Barrier:** On Week 1 Day 3 of every other cycle (Cycles 1, 3 and 5), 24-48 hours post Day 1 FUS treatment with Microbubbles, subjects will undergo MRI with gadolinium pre Day 3 FUS treatment to ensure closure of the blood brain barrier.
j Follow-up MRIs will be conducted every 8 weeks as per SOC as discussed by the study team
k- The subject will be monitored with standard Physical Exam during each cycle and between every cycle.