TITLE: PNOC015: An Open Label Single Arm Phase I/II study of MTX110 delivered by convection-enhanced delivery (CED) in patients with diffuse intrinsic pontine glioma (DIPG) previously treated with external beam radiation therapy

PNOC Protocol #: PNOC-015
Version Number: 2.0
Version Date: 6/20/2019
IND 135229

NCT# 03566199

Revision History

Version 1.0	05/02/2017
Version 1.1	08/26/2017
Version 1.2	10/19/2017
Version 1.3	10/24/2017
Version 1.4	10/31/2017
Version 1.5	12/04/2017
Version 1.6	04/04/2018
Version 1.7	06/25/2018
Version 1.8	10/22/2018
Version 1.9	04/08/2019
Version 2.0	06/20/2019

<table>
<tr><td><u>Study Chair</u>
Sabine Mueller, MD, PhD, MAS
University of California, San Francisco
[redacted]
Email: <u>Sabine.Mueller@ucsf.edu</u></td><td><u>Co-Study Chair</u>
Cassie Kline, MD, MAS
[redacted]
<u>Cassie.Kline@ucsf.edu</u></td></tr>
<tr><td colspan="2"><u>Surgical Study Chairs</u></td></tr>
<tr><td>Nalin Gupta, MD, PhD
[redacted]</td><td>Mark Souweidane, MD
[redacted]</td></tr>
<tr><td colspan="2"><u>PNOC Co-Project Leader</u>
Michael Prados, MD
University of California, San Francisco
[redacted]</td></tr>
<tr><td><u>Coordinating Center</u>
Pacific Pediatric Neuro-Oncology Consortium
Sandler Neurosciences Building
[redacted]</td><td><u>Statistician</u>
Annette Molinaro, PhD
UCSF Department of Neurosurgery
[redacted]</td></tr>
<tr><td><u>Study Nurses</u>
Shannon Raber, RN, PNP, MS
[redacted]
Carly Hoffman, RN, MS, CPNP-AC, CPON
[redacted]</td><td></td></tr>
</table>

Pacific Pediatric Neuro-Oncology Consortium Institutions

Ann & Robert H. Lurie Children's Hospital of Chicago PI: Angela Waanders, MD, MPH Co-PI: Stewart Goldman, MD [redacted]	Children's Hospital Los Angeles PI: Ashley Margol, MD Co-PI: Nathan Robison MD [redacted]
Children's National Medical Center PI: Lindsay Kilburn, MD Co-PI: Roger Packer, MD [redacted]	Dana-Farber Cancer Institute Harvard University PI: Susan Chi, MD Co-PI: Daphne Haas-Kogan, MD [redacted]
Doernbecher Children's Hospital Oregon Health & Science University PI: Kellie Nazemi, MD Co-PI: Rebecca Loret de Mola, DO [redacted]	Johns Hopkins Hospital PI: Kenneth Cohen, MD MBA Co-PI: Eric Raabe, MD [redacted]
Nationwide Children's Hospital PI: Mohamed AbdelBaki, MD Co-PI: Jonathan L. Finlay, M.B. Ch.B. F.R.CP. [redacted]	Rady Children's Hospital University of California, San Diego PI: John Crawford, MD, MS Co-PI: Jennifer Elster, MD [redacted]
Seattle Children's Hospital University of Washington, Seattle PI: Sarah Leary, MD Co-PI: Nicholas Vitanza, MD [redacted]	St. Jude Children's Research Hospital PI: Amar Gajjar, MD Co-PI: Christopher Tinkle, MD [redacted]

St. Louis Children's Hospital Washington University in St. Louis PI: Karen Gauvain, MD, MSPH Co-PI: Josh Rubin, MD, PhD [redacted]	Texas Children's Hospital PI: Patty Baxter, MD Co-PI: Will Parsons, MD PhD [redacted]
The Children's Hospital of Philadelphia PI: Jane Minturn, MD PhD Co-PI: Kristina Cole MD, PhD [redacted]	The Hospital for Sick Children PI: Eric Bouffet, MD Co-PI: Annie Huang, MD PhD [redacted]
UCSF Benioff Children's Hospital University of California, San Francisco PI: Sabine Mueller, MD, PhD, MAS Co-PI: Cassie Kline, MD, MAS [redacted]	University of Florida PI: Sri Gururangan, FRCP Co-PI: Duane Mitchell, MD PhD [redacted]
University of Minnesota and Children's Hospital Minnesota PI: Chris Moertel, MD Co-PI: Anne Bendel, MD [redacted]	University of Utah PI: Nicholas Whipple, MD, MPH Co-PI: Carol Bruggers MD [redacted]

Collaborating Sites:

Memorial Sloan Kettering Cancer Center
PI: Mark Souweidane, MD
[redacted]

UCSF Benioff Children's Hospital Oakland (in connection with UCSF Benioff Children's Hospital San Francisco)
PI: Tabitha Cooney, MD
[redacted]

Massachusetts General Hospital (in connection with DFCI)
PI: David Ebb, MD
[redacted]

PROTOCOL SIGNATURE PAGE

Protocol No.: PNOC 015 **Version Date:** 06/20/2019

1. I agree to follow this protocol version as approved by the UCSF Protocol Review Committee (PRC), Institutional Review Board (IRB), and Data Safety Monitoring Committee (DSMC).
2. I will conduct the study in accordance with applicable IRB requirements, Federal regulations, and state and local laws to maintain the protection of the rights and welfare of study participants.
3. I certify that I, and the study staff, have received the requisite training to conduct this research protocol.
4. I have read and understand the information in the Investigators' Brochure (or Manufacturer's Brochure) regarding the risks and potential benefits. I agree to conduct the protocol in accordance with Good Clinical Practices (ICH-GCP), the applicable ethical principles, the Statement of Investigator (Form FDA 1572), and with local regulatory requirements. In accordance with the FDA Modernization Act, I will ensure the registration of the trial on the www.clinicaltrials.gov website.
5. I agree to maintain adequate and accurate records in accordance with IRB policies, Federal, state and local laws and regulations.

Study Chair

_____
Printed Name

_____ _____
Signature Date

Participating Site(s) Pacific Pediatric Neuro-Oncology Consortium Institutions

Principal Investigator

_____
Printed Name

_____
Institution

_____ _____
Signature Date

ABSTRACT

Title	An Open Label Single Arm Phase I/II study of MTX110 delivered by convection-enhanced delivery (CED) in patients with diffuse intrinsic pontine glioma (DIPG) previously treated with external beam radiation therapy
Patient population	Children with newly diagnosed diffuse intrinsic pontine glioma (DIPG), previously treated with focal radiotherapy.
Rationale for Study	The overall median survival of children with DIPG is approximately 9 months, and remains unchanged despite decades of clinical trial research. The only standard of care is focal radiotherapy but essentially all children die of this disease. New therapeutic strategies are urgently needed. One of the potential reasons for failure of treatment is the blood-brain and blood-tumor barriers, which exclude potentially effective therapeutic agents. Direct delivery by convection-enhanced techniques can overcome this barrier and ensure adequate drug exposure to tumor cells. MTX110 is a soluble from of panobinostat. Panobinostat has been shown in pre-clinical models to be effective at slowing tumor growth in patient-derived brainstem xenografts, and these findings were seen both among carriers of histone mutations and wildtype histone models. The hypothesis of this study is that repeated direct delivery via convection-enhanced delivery (CED) of MTX110 will increase progression-free and overall survival in children with newly diagnosed DIPG following standard of care radiotherapy. This trial will assess the safety and preliminary efficacy of this strategy.
Primary Objective	To determine the safety and tolerability of repeated administration of MTX110 co-infused with gadoteridol given by intratumoral CED in children with newly diagnosed DIPG.
Secondary Objective	To determine the clinical efficacy of repeated administration of MTX110 given by intratumoral CED in children with newly diagnosed DIPG in the confines of a phase I/II study.
Exploratory Objectives	• To assess the magnetic resonance image-guided intracranial injection procedure in patients with DIPG by correlating the observed distribution of gadoteridol to pre-treatment modeling of the drug distribution utilizing predictive imaging software. • To assess Quality of Life (QOL) in pediatric patients with newly diagnosed DIPG treated with MTX110 co-infused with gadoteridol given by intratumoral CED. • To perform central review of imaging to explore MR qualitative and quantitative measures as markers of disease response and/or progression and treatment effect in comparison to institutional evaluation of disease response and/or progression.

Study Design	This is an open-label, ascending dose, single arm phase I/II study of MTX110 delivered by CED in patients with DIPG following standard of care focal radiotherapy. We will start with a single dose drug concentration of 30 μM using a total volume of 3mL and administer MTX110 on day 1 only; we will then first dose escalate by the number of days MTX110 is given: 30 μM concentration, 3 ml total volume, administration will occur on day 1 and 2. We then will start to dose escalate the total volume that is being administered on days 1 and 2. Once the RP2D total volume has been determined, we will then escalate the concentration of MTX110 that is being administered on days 1 and 2. The accelerated dose escalation design will allow intra-patient dose escalation. The concentration of gadoteridol (ProHance) will be 0.5 mM which is the same concentration that is being used for other PNOC studies; both agents will be combined and co-infused via the same catheters. A maximum of 2 catheters will be placed.
Number of patients	The number of subjects enrolled will depend upon the DLTs observed and the number of dose levels tested as the study progresses in accordance with an accelerated dose escalation design (ATD) followed by a 3+3 design. The ATD is expected to enroll 1 to 7 subjects with a minimum of 5 patients in the 3+3 design. Additional subjects might be needed if dose de-escalations need to occur. After review of the initial dose levels as well as achieved coverage areas of the tumor, the study team will also assess if additional dose levels should be tested to achieve adequate tumor coverage. In the pilot efficacy study we will enroll a total of 19 evaluable subjects.
Duration of Therapy	Eligible subjects will receive MTX110 after completion of standard focal radiotherapy (minimum of 28 days, maximum of 14 weeks from radiation therapy). Treatment will occur every 4-8 weeks. Treatment will continue for up to 24 months or until tumor progression or intolerable toxicity. If subjects continue to derive clinical benefit, discussion with investigators and study sponsor will determine feasibility and clinical appropriateness to continue therapy beyond 24 months.
Duration of Follow up	All subjects will be followed for safety, time to tumor progression and overall survival.
Duration of study	The study will reach completion approximately 3 years from the time the study opens to accrual.
Study Drugs	MTX110 is a water soluble formulation of panobinostat, administered via CED; concentration of gadoteridol (ProHance) will be 0.5 mM in all dose levels; both agents will be combined and co-infused via the same catheters.

Safety Assessments	We will use CTCAE version 4.0 for adverse event reporting; surgical complications will be graded as outlined in the protocol. Subjects will undergo clinical evaluation and brain MR imaging prior to any CED to assess for related toxicity, as well as tumor status. Toxicity is defined per standard pediatric phase I guidelines including interruption of planned therapy > 10 weeks to allow for recovery from toxicity of prior CED MTX110 injection.
Efficacy Assessments	We will assess the preliminary efficacy using overall survival at 12 months (OS12) in 19 evaluable DIPG patients treated within the expansion cohort which will include subjects treated as part of the dose escalation portion of the trial on the recommended phase 2 dose (RP2D). The most recent Children's Oncology Group study that treated children newly diagnosed with DIPG with a combination of radiation therapy and temozolomide resulted in an OS12 rate of 40% (SD ± 6.5%). A sample size of 19 patients achieves 80% power to detect a difference of 30% using a one sided exact binominal test. The target significance level is 5% and the actual significance level is 3.5% with this test. If 12 or more patients are alive at 12 months, the null hypothesis that OS12 is 40% will be rejected.
Unique Aspects of this Study	This is the first study to evaluate the safety and preliminary efficacy of the use of repeated CED of MTX110 with real time gadoteridol imaging in children with DIPG.

EXPERIMENTAL DESIGN SCHEMA

Newly Diagnosed Diffuse Intrinsic Pontine Glioma

Phase I Study

Open to patients 2 years to less than or equal to 21 years of age with newly diagnosed DIPG who have completed focal radiotherapy (to begin treatment ≥ 28 days to ≤ 14 weeks from completion of radiation therapy). Enrollment occurs after completion of radiation therapy.

We will assess an anticipated 7 dose levels using an accelerated, intra-patient dose escalation scheme:

- **Dose level 1**: Single CED of 30 μM MTX110 on 1 day (day 1); Total volume 3 mL
- **Dose level 2**: Repeated CED of 30 μM MTX110 on 2 consecutive days (days 1, 2); Total volume 6 mL (3 mL on each day)
- **Dose level 3**: Repeated CED of 30 μM MTX110 on 2 consecutive days (days 1, 2); Total volume 8 mL (4 mL day on each day)
- **Dose level 4**: Repeated CED of 30 μM MTX110 on 2 consecutive days (days 1, 2); Total volume 10 mL (5 mL day on each day)
- **Dose level 5**: Repeated CED of 30 μM MTX110 on 2 consecutive days (days 1, 2); Total volume 12 mL (6 mL day on each day)
- **Dose level 6:** Repeated CED of 60 μM MTX110 on 2 consecutive days (days 1, 2); Total volume 12 mL (6 mL day on each day)
- **Dose level 7:** Repeated CED of 90 μM MTX110 on 2 consecutive days (days 1, 2); Total volume 12 mL (6 mL day on each day)

Once these dose levels have been assessed, a detailed review of the safety and efficacy data will be performed and the study team will discuss with the FDA and the DSMC to determine either further dose escalation or enrollment into the phase II study.

**Of note, timing of follow-up day 2 infusion will be determined pending patient's return to pre-infusion baseline neurological exam or equal or less than grade 1 toxicities, but will occur no later than 48 hours after completion of infusion on day 1; if patient's neurological exam has not returned to pre-infusion baseline by 48 hours from completion of day 1 infusion, day 2 CED procedure will be canceled.

Phase II Study (Expansion Cohort)

A total of 19 subjects will be enrolled to assess the clinical efficacy of the RP2D within the confines of this pilot study.

LIST OF ABREVIATIONS

AA	Anaplastic astrocytoma
AE	adverse event
ALP	alkaline phosphatase
ALT	alanine aminotransferase
ANC	absolute neutrophil count
AST	aspartate aminotransferase
ATD	Accelerated titration design
AUC	area under the curve
BUN	blood urea nitrogen
CBC	complete blood cell (count)
CED	Convection-Enhanced Delivery
CNS	Central Nervous System
CR	complete response
CRC	Clinical Research Coordinator
CRF	case report form
CSF	cerebral spinal fluid
CT	computerized tomography
CTCAE	Common Terminology Criteria for Adverse Events
DFS	disease-free survival
DIPG	Diffuse intrinsic pontine glioma
DLT	dose limiting toxicity
DSMC	Data and Safety Monitoring Committee
DSMP	Data and Safety Monitoring Plan
EGFR	Epidermal growth factor receptor
EFS	Event-free survival
EIAED	enzyme-inducing anti-epileptic drug
FDA	Food and Drug Administration
GBM	Glioblastoma multiforme
GCP	Good Clinical Practice
GDL	Gadolinium liposomes
HAT	Histone acetylase
HDAC	Histone deacetylase
HDFCCC	Helen Diller Family Comprehensive Cancer Center

HGB	Hemoglobin
HGG	High grade glioma
IND	investigational new drug application
IP	investigational product
IRB	Institutional Review Board
IT	intratumoral
IV	intravenous
MedDRA	Medical Dictionary for Regulatory Activities
MRI	magnetic resonance imaging
MTD	maximum tolerated dose
NCI	National Cancer Institute
OR	Operating room
ORR	overall response rate
PD	Progressive disease
PFS	Progression-free survival
PK	Pharmacokinetics
PO	*Per os* (by mouth, orally)
PR	partial response
PRC	Protocol Review Committee (UCSF)
QoL	Quality of Life
RCD	Real-time convective delivery
RP2D	Recommended phase 2 dose
SD	stable disease
SD	standard deviation
SGPT	serum glutamic pyruvic transaminase
ULN	upper limit of normal
Vd	Volume of distribution

TABLE OF CONTENTS

1. OBJECTIVES

1.1 Primary Objective

To determine the safety and tolerability of repeated administration of MTX110 co-infused with gadoteridol given by intratumoral convection enhanced delivery in children with newly diagnosed DIPG.

1.2 Secondary Objective

To determine the clinical efficacy of repeated administration of MTX110 given by intratumoral CED in children with newly diagnosed DIPG in the confines of a phase I and early efficacy study.

1.3 Exploratory Objectives

To assess the magnetic resonance image-guided intracranial injection procedure in patients with DIPG by correlating the observed distribution of gadoteridol to pre-treatment modeling of the drug distribution utilizing predictive imaging software.

To assess Quality of Life (QOL) in pediatric patients with newly diagnosed DIPG treated with MTX110 co-infused with gadoteridol given by intratumoral CED.

To perform central review of imaging to explore MR qualitative and quantitative measures as markers of disease response and/or progression and treatment effect in comparison to institutional evaluation of disease response and/or progression.

2. BACKGROUND

2.1 Convection Enhanced Delivery

CED is a delivery modality which utilizes bulk flow, or fluid convection, established as a result of a pressure gradient rather than a concentration gradient [1]. Through the maintenance of such a pressure gradient from the delivery cannula tip to the surrounding tissues, CED is able to distribute small and large molecules to clinically significant target volumes (centimeters rather than millimeters) [1, 2]. Small or large particles are easily distributed within the brain via CED [3, 4]. The advantages of CED over diffusion based delivery include: (i) an expanded volume of distribution (Vd); (ii) a uniform concentration of the infused therapeutic within the target Vd; (iii) the delivery of the vast majority of the infused therapeutic within the target volume [2].

CED distribution is enhanced by the arterial pulsations within the brain's perivascular spaces [5]. Additionally, an improved understanding of the complexities of the extracellular matrix and its effects on convection has led to better distribution [6-8]. For example, technical CED infusion parameters, such as cannula size and shape, infusion rate (usually 0.2–5.0 μL/min or 0.012–0.3 mL/h), infusate concentration, and tissue sealing time, have been defined and refined to improve distribution of study agents while limiting potential toxicities and morbidities [9-11].

A major advance in the safe and potentially efficacious use of CED in neurosurgery has been the development of real-time convective delivery (RCD), which utilizes magnetic resonance imaging to visualize the CED process with the aid of co-convected contrast agents [11-16]. The use of RCD allows physicians to directly monitor the distribution of therapeutics within the brain. Thus, reflux along the CED catheter or leakage outside the target area, especially at higher flow rates, can be monitored and corrective steps taken, such as retargeting the catheter or altering the rate of infusion [17, 18]. RCD techniques will be used in this study –which represents an important advancement because several recent clinical trials that utilized CED for the delivery of therapeutics to the brain, but which lacked effective imaging monitoring, did not meet clinical endpoints, including trials for treatment of neurodegenerative disease, and neoplastic conditions [19-24].

These clinical trials have demonstrated the promise of CED as a therapeutic option for patients with recurrent high grade gliomas with a relatively low toxicity profile associated with therapy.

Clinical Neuro-Oncologic Studies

Although a large number of therapeutic options have been examined for CED, only a few agents have been formally studied in clinical trials. Some of the more prominent clinical trials studying CED include: TP-38, cintredekin besudotox, IL-4 pseudomonas exotoxin, paclitaxel, AP 12009, and cotara.

Sampson et al. used CED infusion of TP-38 which is a chimeric protein that fuses TGF-α with a genetically modified Pseudomonas exotoxin. Overall survival was 28 weeks with a subset survival of 33 weeks in the 20 patients with no residual disease and 20.1 weeks in patients with residual disease [25]. There was one patient that at the time of publication had a survival of 260 weeks. The toxicities with TP-38 included 5 patients with seizures who had previous seizure disorders. Grade 3 or 4 toxicity was seen in 2 patients [26].

Cintredekin besudotox was tested in 51 patients over the series of three phase I clinical trials for recurrent high grade gliomas. There was an overall survival rate of 45.9 weeks post-treatment, with 9 patients exhibiting progression free survival for 1 year and seven patients with progression free survival of 2 years [27]. There were grade 3 or 4 toxicities reported with headache, convulsion, and hemiparesis; six patients developed hemiparesis. A phase III trial was completed for cintredekin besudotox that was randomized against Gliadel wafers for recurrent GBM. The median survival was comparable between CED and Gliadel wafers at 36.4 weeks and 35.3 weeks respectively. Despite no statistical significance in improved survival for CED-administered therapy, inadequate drug distribution and improper catheter placement might explain the results [28].

A recombinant cytotoxin that is a fusion between IL-4 and Pseudomonas exotoxin was applied by CED in a phase I clinical trial for recurrent gliomas [29]. When comparing the recombinant cytotoxin to resection alone the overall median survival of recurrent GBM was 5.8 months and 5.3 months, respectively [25]. There were no noted systemic toxicities, and the most common adverse events were cerebral edema, seizures, and headaches.

For infusion of paclitaxel for recurrent grade III or IV gliomas, Lidar et al. demonstrated an overall median survival of 7.5 months. However, there was a significant number of treatment related adverse events including transient neurological deterioration from cerebral edema (20%), bacterial infections (15%), and chemical meningitis (30%) [30].

AP 12009, an antisense oligonucleotide that binds to TGF-β2 mRNA to inhibit translation, was studied in three phase I/II clinical trials for CED that demonstrated a median overall survival of 44 weeks for glioblastoma multiforme (GBM) and 146.6 weeks for patients with anaplastic astrocytoma (AA) [31]. There were 29 treatment-related toxicities, but these were mostly minor and limited to grade 2 and 3 toxicities.

Cotara, an immunotoxin with an antibody specific for histone H1-DNA complex that was labeled with ^{131}I, was studied in 51 patients enrolled in phase I and II studies. This treatment achieved a median survival in GBM patients of 37.9 weeks [32]. Eighteen patients had a grade 3 or 4 neurological toxicity, while four patients had grade 3 systemic toxicities [2].

These clinical trials have demonstrated the promise of CED as a therapeutic option for patients with recurrent high grade gliomas with a relatively low toxicity profile associated with therapy.

2.2 Real-time Imaging of Convection Enhanced Delivery (RCD)

The use of RCD in this trial will allow direct visualization of the study drug and improved estimation and standardization of the therapeutic contact time. RCD will be used at the beginning of the infusion and then periodically throughout the infusion, depending on the estimated infusion time for each individual subject. With advancements in magnetic resonance imaging (MRI), the technology now exists to allow for prospective, real-time imaging of the CED injection - which has the potential to ensure more accurate and consistent delivery of the full dose of drug to the tumor. MRI Interventions and BrainLab have developed FDA-approved, head coil, delivery catheters and skull fixation devices that are MRI compatible and allow for real-time imaging of the CED injection. Dr. Russell Lonser at Ohio State University has used the SmartFlow catheter (MRI Interventions) and ClearPoint MRI guided neuronavigation device (MRI Interventions) to deliver a biologic therapy into patients with brain stem glioma. Dr. Mark Souweidane at Memorial Sloan Kettering is using the flexible BrainLab catheter for CED for children with DIPG. The flexible BrainLab catheter allows longer infusion times compared to the SmartFlow catheter system. Gadolinium is added to the study drug to allow for RCD. Prior work has shown that gadoteridol (ProHance®) is biocompatible with the MTX110 and can be safely injected in combination into the tumors of study subjects. Further, Dr. Bankiewicz has shown that this approach is safe and feasible in non-human primates when infused to midline structures of the brain including the brainstem (unpublished data, please see below for more detail). Gadolinium-based contrast agents are not approved for intracerebral administration, although the enhancement seen on standard gadolinium-MRI exams is the result of gadolinium leaking from tumor vessels into the surrounding cerebral tissues. Nonionic gadolinium-based contrast agents have been safely administered intrathecally at 0.5 mL to detect CSF leaks. Gadolinium has also been administered intracerebrally to patients in two studies using CED [16].

ProHance® is a nonionic contrast agent manufactured by Bracco Diagnostics that has been used clinically for many years. The addition of ProHance® to MTX110 has been successfully tested for compatibility. ProHance® contains 279 mg/mL of gadolinium and during a typical intravenous administration, a 70 kg man would receive approximately 3,906 mg of gadolinium. In this study a maximum of 0.03 mL of ProHance® of a 1:10 dilution will be added to 3.0mL of MTX110 yielding <1.0 mg/mL of gadolinium.

Pre-clinical Neuro-Oncologic Studies

CED has been used to effectively deliver therapy within the central nervous system (CNS) in small animals with or without tumors [4, 33-36], canines with spontaneous brain tumors [37, 38], and primates [11, 39]. In canines, similar CED-delivered agents failed to show clinical or pathological adverse effects in normal or brain tumor-bearing animals [37, 38], while confirming clinical efficacy and highlighting the importance of RCD to maximize tumor coverage and minimize inappropriate infusions. Convection of gadolinium liposomes (GDL) in nonhuman primate brain has confirmed the lack of toxicity and ability to monitor the infusion process in a larger brain using RCD methods similar to those for humans [11, 40]. Recently, *in vivo* CED of magnetic nanospheres conjugated to an antibody that selectively binds to the epidermal growth factor receptor (EGFR) mutant (EGFRvIII) found on glioblastoma xenografts, not only allowed specific tumor visualization on MRI, but through an apoptotic mechanism, was associated with targeted cell death with sparing of normal astrocytes [41] .

These and other preclinical data [42] argue for the importance of a delivery platform [17] that utilizes RCD to monitor therapeutic distribution and potential complications associated with CED in the brain tumor patient [18].

2.3 Diffuse Intrinsic Pontine Glioma (DIPG), CED, MTX110

Gliomas are the most common brain tumor type in children. High-grade gliomas (HGG), including anaplastic astrocytomas (AA) and glioblastomas (GBM), are the most aggressive gliomas with 5-year survival rates of less than 20% in the pediatric population. If these tumors occur within the pons – diffuse intrinsic pontine glioma (DIPG) – the expected outcomes are even worse, with a median survival of approximately 9 months. Despite several decades of research, outcomes and treatment strategies for these children has not significantly changed and essentially all children die from this disease. For children with DIPG no standard treatment has been established although most children are treated with focal irradiation. Once DIPG recurs or progresses, there are very few treatment options and most children die of their disease within a very short timeframe on the order of months. One prior study reported an event-free survival (EFS) defined as the time from progression to time of death, clinical deterioration or imaging based progression to be 2 months [43]; however published data on progression free survival (PFS) or EFS for progressive DIPGs is limited. Clearly new therapies and strategies are needed.

One of the reasons for treatment failure is the presence of an intact blood-brain and blood-tumor barrier, with tight packing of cellular structures within the brainstem. These anatomic barriers are a significant obstacle to the effective delivery of many small-molecule drugs and most biological therapeutics. As mentioned above, CED utilizes a pressure gradient to distribute pharmacologic agents by bulk flow of fluid through tissue interstitial spaces. CED markedly improves drug

distribution within the brain when compared with non-CED injections or the implant of drug eluting polymers, both of which rely on passive diffusion. Additionally, CED results in far greater drug concentration while minimizing systemic exposure and toxicity. Here, we propose a phase I clinical trial to assess the safety and early efficacy of repeated CED infusion of MTX110 in children with DIPG.

Histone modifier mutations drive tumorigenesis

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 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-kB 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 (a-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[44].

Targets of MTX110, Histone 3.3 and 3.1 K27M mutations, are expressed in DIPGs

The most prevalent molecular alterations of pediatric HGGs involve recurrent alterations of genes regulating epigenetic modifications of the genome[45, 46]. Genome-wide studies have identified recurrent hotspot mutations in histone H3, family 3A (*H3F3A*) and histone cluster 1, H3b (*HIST1H3B*), which encode the histone H3 variants H3.3 and H3.1 respectively. Recent genetic studies have revealed that malignant gliomas in children often show recurrent missense mutations in *H3F3A*, which encodes the replication-independent histone 3 variant H3.3.[45, 47, 48] Approximately 70% of DIPG [49] cases harbor a mutation which results in a substitution of the amino-acid lysine (K) to methionine (M) at position 27 of H3.3 (K27M mutation, hereafter), which is associated with shorter survival in DIPG patients compared with patients with non-mutated H3.3[49].This was the first example of a human tumor driven by direct mutation of a histone gene. Point mutations of *H3F3A* and *HIST1H3B* genes occur at hotspots at amino acid residue 27 resulting in lysine to methionine (K27M) amino acid change. Mutations lead to

marked changes of the epigenome resulting in distinct methylation signatures.[50, 51] Recent studies have shown that there also seems to be a difference between the two K27M mutations in that children harboring the H3.1K27M mutation have a worse clinical outcome compared to children with harboring the H3.3K27M.[49] K27M-mutated tumors are associated with the worst survival with a median overall survival of only 12 months.

In vitro pre-clinical models of MTX110

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. Using DIPG cell cultures from 16 patients, Grasso et al screened 83 existing and potential cancer drugs and found that 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 panobinostat and similar gene-regulating drugs may be effective at treating DIPG. Using DIPG cell cultures from 16 patients, Grasso et al screened 83 existing and potential cancer drugs and found that HDAC inhibitors consistently slowed cancer cell growth in DIPG cell lines, and inhibited DIPG growth and extended survival in a DIPG mouse model.[52]

MTX110 was evaluated for HDAC enzyme inhibition and glioma cell cytotoxicity in three studies as shown in Table 1.

Table 1: MTX110 Pharmacology Studies

Midatech Study Reference	**Study Description**	**Notes and Main Findings**
University of Bristol Study	Cytotoxicity in DIPG cells	IC50 = 10nM. SF8628 DIPG cells incubated with MTX110 for 72 hours.
MTX110-V0031T	Cytotoxicity in U87MG cells	IC50 = 10nM. U87MG glioblastoma cells incubated with MTX110 for 48 or 72 hours.
MTX110-V0024P	HDAC enzyme inhibition HDAC enzyme inhibition, cell- based assay	MTX110 and panobinostat DMSO had comparable enzyme inhibiting potency across HDAC 1-11 enzymes (see Table 3 below) MXT110 (10.9nM) and panobinostat DMSO (2.6nM) had similar IC50 values against HDAC Class I/II enzymes.

MTX110 was a highly potent inhibitor of HDAC, with IC50 values for HDAC1-11 enzymes comparable to panobinostat DMSO solutions (Tables 1 and 2). Similarly, MTX110 showed comparable inhibition of HDAC Class I and II enzymes in cell based assays (Table 1).

MTX110 was similarly highly potent as a cytotoxic agent in U87MG glioma and human DIPG cell lines, with an IC50 of 10nM (Table 1). This is consistent with a published study which demonstrated panobinostat to be an effective cytotoxic agent across 14 different DIPG cell lines, with IC50 values in the range 20-2000nM [52]. The same authors demonstrated efficacy of panobinostat.

DMSO solutions in a DIPG xenograft mouse model after intraperitoneal administration at doses of 10 and 20 mg/kg and after CED administration of 2μM panobinostat DMSO solution (4μL/minute) for 12.5 minutes.

Table 2: HDAC Enzyme Inhibition (Study MTX110-V0024P)

IC50 for HDAC enzyme inhibition (nM)										
HDAC 1	**HDAC 2**	**HDAC 3**	**HDAC 4**	**HDAC 5**	**HDAC 6**	**HDAC 7**	**HDAC 8**	**HDAC 9**	**HDAC 10**	**HDAC 11**
Panobinostat (MTX110)										
8.6	16	4.0	567	110	7.5	1820	56	927	20	1380
Panobinostat (DMSO)										
1.6	5.0	0.8	1550	166	1.8	3680	52	1040	13	1670

Use of HDAC inhibition via panobinostat in oncology and in DIPG

Panobinostat (Farydak™) has been approved as a medicinal product for oral use in the US and EU for the treatment of multiple myeloma and has undergone extensive preclinical and clinical safety evaluation to support this indication. No panobinostat product suitable for direct brain delivery by CED is available.

MTX110 is a novel, soluble, panobinostat formulation undergoing experimental evaluation for administration by CED to treat certain brain tumours. The safety evaluation program for MTX110 therefore focused on physicochemical characterisation of the formulation and toxicological evaluation of direct delivery of MTX110 into brain parenchyma by CED.

The studies included: qualification of assays to quantify levels of panobinostat in plasma and brain tissue samples; confirmation of pharmacological potency as an inhibitor of HDAC, and as a cytotoxic agent in glioblastoma and DIPG cells; plasma and brain distribution, and toxicologic evaluation of MTX110 after CED delivery in rats and pigs.

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 refectory 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 clinical evidence that panobinostat does not cross the blood brain barrier when given systemically at tolerated doses. It has been used to reactivate latent HIV in adult patients to facilitate the therapeutic effect of antiretroviral therapy to some following oral panobinostat administration. Panobinostat was not detectable in the CSF of these patients at any time point [53] .

The fact that panobinostat seems to be the most efficacious clinically available drug against DIPG cells, that 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 CED. However, its use is limited by poor water solubility. Drugs need to be water soluble at physiological pH to be delivered to the brain by CED. Midatech Pharma has recently identified a soluble formulation (MTX110) through complexation with hydroxypropyl-β-cyclodextrin that enables CED delivery at potentially chemotherapeutic doses.

Pharmacokinetics of MTX110 delivered via CED in animal models

The nonclinical ADME of panobinostat were established during the development of other panobinostat products. The brain distribution of panobinostat after administration of MTX110 by CED was evaluated in rats and pigs to support clinical CED delivery, as listed in Table 3.

Table 3: MTX110 Pharmacokinetic Studies

Midatech Study Reference	**Study Description**	**Dose (µM)**	**No. Animals**	**Measurements & Observations**
MTX110-R0021K	Rat CED single dose brain distribution study	3 30 300	14 6 6	Plasma and brain panobinostat concentrations were measured (n=2/time) at time 0, 0.5, 2, 6, 24, 48 and 72 hours (3µM) or 0, 2 and 6 hours (30 and 300µM) after CED into the brainstem (3µM) or striatum (30 and 300µM). MTX110 was cleared from the brain with a $t_{1/2}$ of approximately 3 hours.
MTX110-S0023T	Pig CED single dose brain distribution study	30	1 (0d)	One pig was dosed by CED into the striatum and thalamus and sacrificed at Time 0 for brain panobinostat distribution analysis (as part of the toxicology study). The distribution of MTX110 in brain tissue followed the infusion distribution predicted by the MRI planning software.

Three groups of male Wistar rats were treated with MTX110 by CED with as shown in Table 4.

Table 4: Rat CED Pharmacokinetic Study Design

Group No.	**Group Name**	**Dose concentration (µM panobinostat)**	**Sampling time points (2 rats/time point)**
1	MTX110-low	3	0, 0.5, 2, 6, 24, 48, 72 hours after infusion
2	MTX110-inter	30	0, 2, 6 hours after infusion
3	MTX110-high	300	0, 2, 6 hours after infusion

For Group 1 rats (3 µM), the catheter tip was placed stereotactically into the brain stem. The solution was administered at a CED infusion rate of 1 µL/minute for 5 minutes (5 µL volume of infusion). For Group 2 rats (30 µM), and Group 3 rats (300 µM) the catheter tip was placed into the left striatum. Solutions were administered at a CED infusion rate of 2.5 µL/minute for 4 minutes (10µL volume of infusion).

Brain panobinostat concentrations are shown in Table 5. Concentrations were dose-related but increased sub-proportionally with increasing dose. Peak brain concentrations in the infused hemispheres of 1.9, 3.4 and 36.6 ng/g were measured for the 3, 30 and 300 µM solutions, respectively. If it is assumed that all drug is located within the convected tissue (assumed to be 3x the infusion volume), the peak concentrations equate to concentrations in the convected tissue of 253, 227 and 2,440 ng/g, equating to approximately 0.7, 0.7 and 7 µM. At the 300 µM dose, brain concentration declined with time after convection with a tentative half-life of 2 to 3 hours.

Table 5. Brain panobinostat concentrations after CED administration of MTX110 in rats

	Brain concentration (ng/g)					
	Dose 3µM		**Dose 30µM**		**Dose 300µM**	
Time (h)	**Brain Stem**	**Brain**	**Right Brain**	**Left Brain**	**Right Brain**	**Left Brain**
0 hours	BLQ	1.58	BLQ	BLQ	36.6	BLQ
0 hours	1.07	1.97	BLQ	BLQ	20.9	BLQ
2 hours	0.76	1.81	3.41	BLQ	15.0	BLQ
2 hours	BLQ	0.87	2.67	BLQ	19.8	BLQ
6 hours	BLQ	0.86	BLQ	BLQ	6.53	BLQ
6 hours	BLQ	BLQ	BLQ	BLQ	8.70	BLQ

LOQ = 0.75ng/g

In plasma, very low concentrations of panobinostat were measured in occasional samples (Table

6).

Table 6. Plasma panobinostat concentrations after CED administration of MTX110 in rats

Time	**Plasma concentration (ng/mL)**		
	Dose 3µM	**Dose 30µM**	**Dose 300µM**
0 hours	0.59	BLQ	BLQ
0 hours	BLQ	BLQ	BLQ
2 hours	0.28	BLQ	BLQ
2 hours	BLQ	0.66	BLQ
6 hours	BLQ	1.06	BLQ
6 hours	BLQ	0.88	0.643

LOQ = 0.25 ng/mL (3µM dose); 0.5ng/mL (30 and 300µM doses)

A single Large White (Landrace) pig was treated with MTX110 by CED as shown in Table 7.

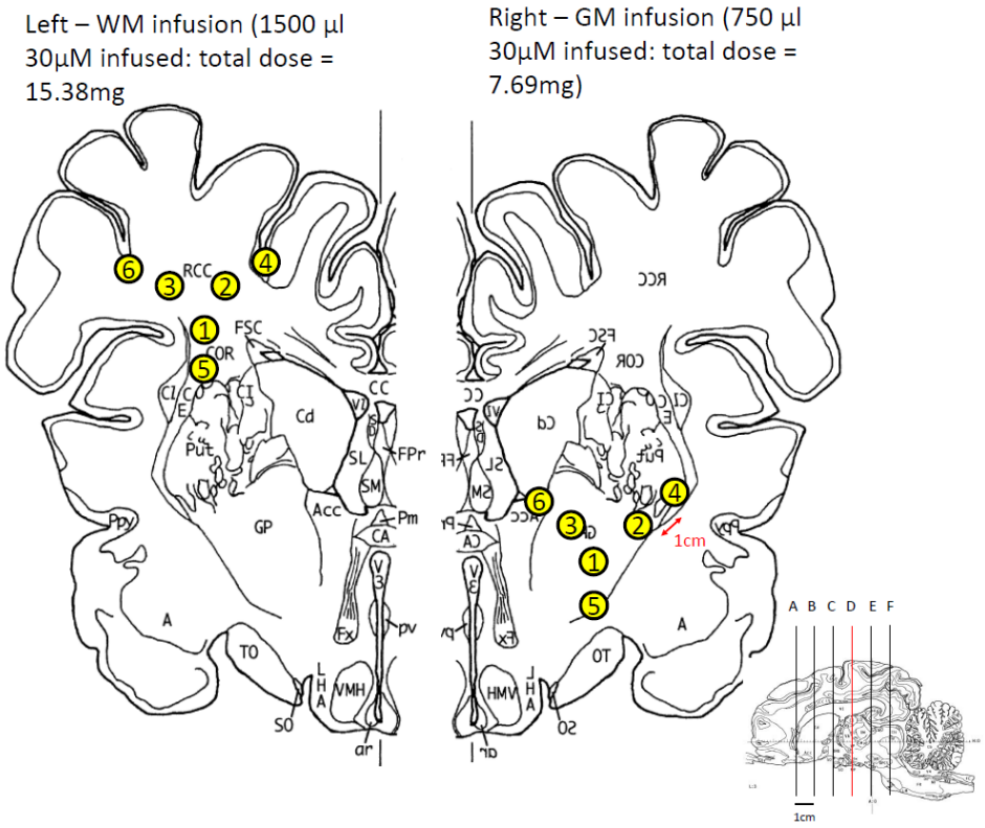
Two catheters were implanted, one into white matter (striatum, left hemisphere) and one into grey matter (thalamus, right hemisphere) using stereo-guidance. A recessed-step catheter design attached to subcutaneous ports was used for this study [54](supplied by Renishaw). The accuracy of catheter targeting was verified by post-operative MRI.

Infusion of MTX110 30µM (10.47 µg panobinostat/mL) was performed by gradually ramping up the infusion rate, monitoring in real-time with MRI. Serial T2/FLAIR imaging was performed during infusions to allow monitoring of infusate distribution. Immediately on completion of CED infusion, the pig was euthanized for drug distribution analysis. A map showing the areas sampled is shown in Figure 1 below for one of 6 brain slices of 1cm thickness (as shown in the inset diagram). The sampling pattern was replicated for each of the 6 slices. Sample numbers 1-3 form an inner-perimeter around the catheter tip, and sample numbers 4-6 form an outer perimeter.

Table 7. Pig CED Pharmacokinetic Study Design

Animal Number	**Brain Region Convected**	**Day of termination**	**Infusion Volume (µL)**	**Panobinostat Dose (**µM)
MTX110 (Pig No. 4)	White matter (left)	Day 0	1500µL	30µM
	Grey matter (right)		750µL	30µM

Figure 1. Punch biopsy sampling sites in Pig 4



Brain panobinostat concentration data are shown in Table 8 for each of the sample numbers and slice letter codes shown in Figure 1. Drug was measurable in several regions away from the catheter location indicating successful convection of the dose. The average peak concentration was 1,042 ng/g, which is equivalent to approximately 3 µM.

Table 8. Brain panobinostat concentrations for punch biopsy samples in Pig 4 (see Figure 1 for sample map)

	Left Brain Slice Letter Code (ng panobinostat/g)						Right Brain Slice Letter Code (ng panobinostat/g)					
	A	B	C	D	E	F	A	B	C	D	E	F
1	BLQ	BLQ	49.8	35.9	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	67.3	BLQ
2	7.79	BLQ	19.2	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	5.98	52.0	BLQ
3	BLQ	BLQ	6.70	9.63	BLQ	BLQ	BLQ	BLQ	BLQ	433	BLQ	BLQ
4	BLQ	5.40	1650	71.5	BLQ	BLQ	BLQ	BLQ	BLQ	19.4	BLQ	BLQ
5	BLQ	BLQ	BLQ	6.60	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	11.1	BLQ
6	BLQ	BLQ	197	1.42	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ

Safety of MTX110 given via CED in animal models

The brain toxicity of MTX110 was evaluated in rats and pigs treated using CED in two studies, as listed in Table 9.

Table 9: MTX110 Pharmacokinetic and Toxicity Studies

Midatech Study Reference	**Study Description**	**Dose (µM)**	**No. Animals**		**Measurements & Observations**
			72h	**14d**	
MTX110-R0022T	Rat CED Single Dose CNS Toxicity Study	0 (aCSF)	3	3	Rats were dosed by CED into the brainstem and sacrificed 72 hours or 14 days after dosing. Brains were examined in detail for histopathological changes and no drug-related histophatologic changes were seen.
		0 (HPBCD)	3	3	
		0.3	3	3	
		1	3	3	
		3	3	3	
		10	3	3	
		30	3	3	
MTX110-	Rat CED		**7d**	**14d**	Rats were dosed by

R0226T	Single Dose CNS Toxicity Study	0 (Saline) 30 100 300	4 4 4 4	- - - -	CED into the brainstem and sacrificed 7 days after dosing. Brains were examined in detail for histopathological changes. The maximum non-neurotoxic concentration of MTX110 was determined to be > 300 μM.
MTX110-R0227T; MTX110-R0197T	Rat CED Single Dose CNS Toxicity Study	0 (Saline) 1000 3000	**7d** 4 4 4	**14d** - - -	Rats were dosed by CED into the striatum and sacrificed 7 days after dosing. Brains were examined in detail for histopathological changes. The maximum non-neurotoxic concentration of MTX110 was determined to be about 1000 μM.
MTX110-S0023T	Pig CED Single Dose CNS Distribution and Toxicity Study	0 30 30	**7d** - 1 -	**14d** 1 - 1	Pigs were dosed by CED into the brainstem and sacrificed 7 days or 14 days after dosing. Brains were examined in detail for histopathological changes and no drug-related histophatologic changes were seen.

aCSF – artificial cerebrospinal fluid; HPBCD – hydroxypropyl-β-cyclodextrin*Rat Studies*

Six groups of male Wistar rats were treated with MTX110 by CED as shown in Table 10.

Table 10. Rat CED Toxicity Study Design

Group No.	Group Name	Dose (μM Panobinostat)	No. Rats (72 hours)	No. Rats (14 Days)
1	aCSF Control	0	3	3
2	HPβCD Control	0	3	3
3	MTX110 - HIGH-2	30	3	3
4	MTX110 - INTER-1	1	3	3
5	MTX110 - INTER-2	3	3	3
6	MTX110 - HIGH	10	3	3

Test and control solutions were administered to the rats by CED. A catheter tip was placed stereotactically into the brain. Solutions were administered at an infusion rate of 1 μL/minute for 5 minutes (5 μL volume of infusion). Target accuracy was confirmed with infusion of dye into the brain stem of one additional animal using the same infusion parameters, and visualized at 0 hours post infusion. Either 72 hours or 14 days after dosing, the animals were killed for histopathological examination of the brain.

The rats tolerated CED treatment well, and there were no adverse in-life observations. One rat treated at 30μM was found dead the morning after dosing with no cause of death established. In other rats, intraventricular administration of MTX110 was well tolerated. Histopathological examination revealed no toxicity and the catheter damage associated with the route of administration was limited and repaired readily. At 72 hours, the initial points of catheter entry were visible in the majority of animals. Lesions at the point of entry were in the visual cortex in the region of the pineal gland, and comprised focal necrosis with astrocytosis, macrophage infiltrates and haemorrhage. The largest lesion was in an a CSF control, and extended to the superior colliculus; there was also a linear lesion in neuropil near the aqueduct. Similar linear lesions in the neuropil close to the aqueduct were seen in several animals. Similarly, there were linear lesions comprising focal vacuolation and astrocytosis with a macrophage infiltrate in the region of the cerebellar carbuncle and extending down towards the pontine nuclei. This area is very close to the 4th ventricle, an area that would have the potential to be a region where a relatively high concentration of the test item could be expected, but given the lack of a dosage relationship (the lesion was most pronounced in the animals given 1.0 μM MTX110), it was likely that this lesion was also procedural in nature.

One rat in each of the 1 and 3 μM groups had small focal lesions of linear nature in the cerebellar lobules. These were similar in appearance with a localized loss of neurons, a macrophage infiltrate and a minimal astrocytosis and were consistent with localized trauma from the procedure; they were also considered to represent localized damage in the region of the needle path. Immunohistochemical (IHC) stains clarified the nature of the lesions in the visual cortex and the other areas that were considered to be traumatic. The appearance was one of a focal loss of neurons, with the area having been populated by macrophages and a reactive astrocytosis. The IHC stains indicated the lesions were a little more extensive than first appeared on the H and E sections. The appearance strongly suggested the lesions were related to the procedure following a well-recognised pattern for traumatic lesions in the CNS.

Dark field examination with a fluorescent microscope of the sections stained with Fluorjade B

revealed the occasional necrotic neuron in some animals. These were seen within the boundaries of the lesions that resulted from the trauma associated with the route of administration, and when present only occurred at an incidence of one or two per field. There was no evidence of any neuronal damage resulting from the presence of Panobinostat. There were no changes seen at the highest dose that were not present in the other dose groups. As such it was considered that at 72 hours there were no toxic changes associated with the administration of MTX110. After 14 days, the initial points of entry were still visible in the majority of the animals. The lesions had a similar appearance but showed signs of resolution. No new lesions had developed in the period between the sacrifices, and the lesions that were associated with the route of administration were generally smaller and clearly resolving.

Pig Studies
Three Large White (Landrace) pigs were treated with MTX110 by CED or left untreated as shown in Table 11. The catheter type and placement mimicked those to be used in the proposed protocol.

Table 11. Pig CED Toxicity Study Design

Animal Number	Brain Region Convected	Day of termination	Infusion Volume (µL)	Panobinostat Dose (µM)
Toxicology:				
MTX110 (Pig 1)	PONS	Day 7	100µL	30µM
MTX110 (Pig 2)	PONS	Day 14	100µL	30µM
Control (Pig 3)	Untreated Control	Day 14	N/A	N/A

A single catheter (Renishaw) was implanted into the target area (pons brain stem). The accuracy of catheter targeting was verified by post-operative MRI. Infusion of MTX110 30µM (10.47 µg panobinostat/mL) was performed by gradually ramping up the infusion rate, monitoring in real-time with MRI. Serial T2/FLAIR imaging was performed during infusions to allow monitoring of infusate distribution. The total time of infusion was 47 minutes. If appropriate, the animals were allowed to recover from anaesthesia. The pigs were euthanized by terminal anesthesia and the brain was explanted. The brain was orientated optimally for sectioning along the CED catheter tracks, embedded in paraffin wax, and sectioned to generate 9 sections of brain (12 samples) for each pig. Sections were stained with haematoxylin and eosin for histopathologic examination, and special stains were used to assess damage to specific cell types as follows: NeuN (neuronal cell specific marker), GFAP (glial fibrillary acidic protein, a glial cell specific marker), Fluorojade (neuronal degeneration stain) and IBA-1 (a microglia marker).

Both MTX110-treated toxicology pigs had some hind leg weakness following recovery from surgery. Pig 2 recovered quickly over 24 hours, whereas Pig 1 had weakness in the right leg for 2 days and in the left leg for 5 days after treatment. Hind edema was also noted in Pig 1, which partially responded to administration of non-steroidal analgesia. After detailed consultation with a veterinarian, the weakness was attributed to surgical positioning and prolonged anaesthesia

(approximately 6 hours) in this pig and not to MTX110, as such effects have been observed previously in animals undergoing extended anaesthesia. Eating and drinking in both animals was normal. There were no physiological signs of brain stem dysfunction in either pig.

Histopathological examination of the pig killed after 7 days clearly showed the needle track in the sections, which included the thalamus and the pons. At this time-point there was haemorrhage and a cellular response that was GFAP negative and IBA-1 positive, indicating a macrophage/microglial response with no apparent astrocytosis (Figure 2). A little perivascular cuffing was seen in the region of the needle track in the sections of the pons. The amount of haemorrhage appeared to be more than was seen in the rodent study (MTX110-R0022T).

There were occasional vacuoles containing myelin bodies adjacent to the areas of the needle track, a finding indicative of some previous neuronal loss. This was particularly evident in the section from the distal pons where there was a focal lesion but no clear needle track, probably an indication that this was the site of delivery of the test item. However, the changes were not markedly worse than in the other areas of the needle track, and are consistent with the pathology expected from the procedure.

In the animal euthanized after 14 days, the needle track was still clearly visible in the sections that included the thalamus and the pons. At this time-point the haemorrhage had largely resolved, and the cellular response remained GFAP negative and IBA-1 positive. Many macrophages/microglia could be seen to be containing pigment, presumed to be hemosiderin.

No new lesions developed in the period between the sacrifices, and the lesions that were associated with the route of administration were generally smaller and clearly resolving.

Examination of the sections stained with Fluorojade indicated that there was no active necrosis or apoptosis in either of the treated animals. There were no microscopic findings recorded in the control animal.

The route of administration therefore appeared to be well-tolerated, and the lesions caused by the procedure appeared to be resolving readily over the period of the study. The damage that was seen to have occurred was limited to the needle track and the area immediately adjacent to it. The NeuN staining indicated that the path of the needle was in the main passing through areas of low neuronal density, and as such the neuronal loss seen in the area of the track probably represents a very small loss of neurons in numerical terms, and an insignificant loss in biological terms.

In conclusion, the intracranial administration of MTX110 was well-tolerated. There was no toxicity associated with the administration of the test item and the damage associated with the route of administration was limited and showed signs of repair in the time course evaluated.

Safety of MTX110 formulation

All excipients used in the formulation of MTX110 are widely used pharmaceutical ingredients that have established safety profiles in the concentrations used. Hydroxypropyl-β-cyclodextrin (HPBCD) is commonly used to improve the solubility of drugs for intravenous use, but has not previously been delivered directly to the brain by CED. However, HPBCD is administered at very high intravenous doses as a component of some marketed drug products, and compassionate use in children with Niemann Pick Type C has involved intrathecal delivery of high doses

(200mg) to the brain that appear to be well tolerated. Matsuo et al (2014) delivered doses up to 200mg (10mg/kg) or 300mg (15mg/kg) to a single patient (20kg child weight assumed) twice weekly for 23 months[55]. This was reported to be well tolerated although the 300mg dose was stopped when an elevated CSF tau level was detected. Maarup et al (2015) delivered 200mg HPBCD intrathecally to a 12-year old child with NPC, with only subclinical hearing loss at high frequency noted.[56] In an on-going NIH clinical trial, doses of 50 to 400mg HPBCD have been administered to 12 NPC patients intrathecally and are also reported to be well tolerated, except that Grade 1 ototoxicity has been observed in 2 patients.

A dose of 200mg is more than 160 times higher than the dose of HPBCD that would be delivered in MTX110 panobinostat solution for infusion by CED, assuming a convection volume of 12mL of 10μM panobinostat in 100μg HPBCD/mL solution.

Flow rate assessment of CED in non-human primates

Two non-human primates (NHP) were treated with CED of gadolinium (Dr. K Bankiewicz, personal communication). Once the target was selected, infusion of 2mM MR-visible tracer (ProHance, Bracco) was performed by CED by means of small (16G) on the left and large (14G) cannula on the right side of the brain. After visualization of gadolinium infusion at the cannula tip, the infusion rate was ramped up from an initial 1 (16G cannula) or 4 μL/min (14G cannula) to a maximum of 33 μL/min. NHP1 received an infusion on the left thalamus with the 16G cannula and on the right thalamus and brainstem with the 14G cannula. NHP2 was infused bilaterally in the thalamus with the 14G cannula. The total infusion volume per hemisphere was 245 and 1090 μL on left or right side respectively for one animal (NHP1, left thalamus: 246 μL; right thalamus: 438 μL; right brainstem: 655 μL) and 340 μL for the other (NHP2) in each thalamus. There was no observed toxicity that was related to the infusion with the respective volumes and infusion rates.

CED experience in children with DIPG

There are several studies that support the feasibility of placing catheters safely into the brainstem and obtaining adequate coverage of a therapeutic agent using CED in humans. A feasibility study using co-infused imaging tracers with interleukin-13-Pseudomonas exotoxin in children with DIPG has shown that clinically relevant distributions of agents can be achieved in humans through the use of CED [57]. In a recent report of 2 children with DIPG and one patient with Parkinson's disease, Chittiboina et al. showed that cannulas for CED can be accurately placed under direct MRI guidance using a similar navigation system proposed for this study [58]. The same group had previously shown that real-time image guided CED of therapeutic agents is feasible in children with brainstem lesions [16]. A case report by Saito et al. demonstrates a clinical response to a brainstem located high grade glioma to CED of nimustine hydrochloride [59]. Another case report demonstrated safe and effective delivery of carboplatin using CED in a 5 year old with DIPG [60]. The technical feasibility of CED in the brainstem is also supported by a report from Anderson et al. that used CED of topotecan for the treatment of 2 children with DIPG [61]. The first patient underwent CED 210 days after initial diagnosis and completion of radiation therapy at time of tumor progression. A total dose of 0.403 mg topotecan in 6.04 ml over 100 hours was administered. The second patient was treated 24 days after the initial diagnosis prior to radiation with a total dose of 0.284 mg in 5.30 ml over 100 hours.

With respect to volume of distribution, other groups have shown that coverage of the total tumor volume appears to be safe in children with DIPGs. At the recent meeting of the International Society Pediatric Neuro-Oncology (ISPNO, 2016 Liverpool), Dr. Steven Gill (Bristol University, UK) presented results following CED with carboplatin in children with DIPG. The range of infusion volumes and infusion rates (using four catheters) was 2.76 to 11.22 ml and 9-20 µl/min, respectively. Some subjects developed transient neurological symptoms such as weakness, ataxia and dysarthria, but overall, the treatment was well tolerated.

Dr. Mark Souweidane (Memorial Sloan Kettering Hospital, New York City; Surgical co-study chair on this proposal) is currently conducting a clinical trial using CED with a radiolabeled antibody for children with DIPG (NCT 01502917). The majority of the tumor volume can be covered with infusion volumes of 4000 µL without significant adverse effects In this study, repeated CED was an option if the patient was without clinical or radiographic evidence of disease progression. There were no serious adverse events definitively related to surgery in children undergoing retreatment. The lower age limit is 2 years for this trial further supporting this age cut off in the current protocol. Dr. Souweidane is also utilizing repeated CED every 4 weeks after the initial infusion with no safety concerns reported to date[62].

Human experience of CED of MTX 110

The preclinical safety of MTX110 Panobinostat Solution for Infusion has been evaluated by Midatech in collaboration with Bristol University to support experimental use by CED administration on a compassionate named patient supply basis. Patient treatments involved CED administration of MTX110 on one or two consecutive days at doses between 10 and 30 µM panobinostat; all patients treated to date are listed in the table below (Table 12). Further, UCSF conducted a single patient IND trial using MTX110 in a child with progressive DIPG. The CED procedure completed in the compassionate use protocol uses the same BrainLab navigation and catheter system and similar intra-operative MRI monitoring proposed in the current protocol. The patient underwent 2 cycles of CED treatment with MTX110. Both cycles included placement a single catheter and were deemed tolerable. In the first cycle, the patient received a total of 1.5mL of drug infusion and experienced transient grade 1 sinus tachycardia and hypertension in the post-operative period. The patient went on to experience grade 1 dysphagia, grade 2 gait disturbance, and grade 1 abducens and oculomotor nerve disorder a few weeks after hospital discharge. Imaging done at time of new symptoms was concerning for tumor progression; however, contribution of local inflammation from CED therapy could not be ruled out and patient was started on increased dexamethasone for symptom management. In the second cycle, the patient received a total of 4.2mL of drug infusion. After this cycle, the patient experienced grade 2 respiratory disorder due to lung atelectasis in the post-operative disorder, worsening left-side weakness, and grade 3 dysphagia. All symptoms were improving at time of hospital discharge on post-operative day 8. During both CED cycles, the patient received prophylactic dexamethasone to decrease local edema. Dexamethasone was tapered appropriately over hospital course with each cycle. During cycle 2, the patient also received a 24-hour course of mannitol in effort to decrease any component of edema that was contributing to increased dysphagia and weakness. The patient remains alive at the time of this protocol. Overall, these experiences provide supporting evidence of safety and starting dose for the current trial.

Table 12. Pediatric patients treated with MTX110 via CED through compassionate use.

Patient Number	Treating Centre	Number of MTX 110 Doses Received to Date
[redacted]	[redacted]	10
[redacted]	[redacted]	3
[redacted]	[redacted] [1]	1
[redacted]	[redacted] [1]	1
[redacted]	[redacted]	2

[1] from October 2016

Summary of adverse events with cut off date Oct 31, 2018 of the current study.

To date, we have enrolled [redacted] subjects and completed monitoring and DSMC approval of the first 4 dose levels as per the ATD. There were no DLTs at any dose level. Table 13 details related adverse events reported to date.

Table 13. Adverse events probably, possibly or definitely related to protocol therapy:

			Maximum grade across all cycles			
			Grade 1	Grade 2	Grade 3	Grade 4
Toxicity	**Dose level**	**# Evaluable Patients**				
Fatigue	4	1	1	-	-	-

Summary of preclinical and clinical data

In summary, the relevant preclinical and clinical studies support the exploration of CED of MTX110 in children with DIPG:

(1) The target of MTX110 is highly expressed in human DIPGs

(2) Human derived DIPG cell lines are sensitive to MTX110

(3) CED leads to effective distribution within the brainstem

(4) CED of MTX110 under real time imaging is feasible

(5) Published evidence supports the feasibility of CED in children with DIPG

(6) Five total pediatric patients with DIPG receiving MTX110 via CED support safety of delivery of MTX110 via the proposed CED protocol.

2.4 Study Agent

Agent: MTX110 a water soluble formulation of panobinostat for administration via Convection Enhanced Delivery. MTX110 is a lyophilized powder produced under aseptic conditions. The product in reconstituted using normal saline. The appearance of the final solution is a clear

colorless solution.

The composition of each vial of MTX110 is provided below.

Active Ingredient	Function	Amount per 10ml vial
Panobinostat free base	Active	0.14mg
Excipients		
Hydroxypropyl-β-cyclodextrin	Improve solubility	4.00mg
Sodium hydroxide	pH adjustment	0.39mg
Sodium citrate dihydrate	Improve solubility	0.21mg
Citric acid	Improve solubility	0.64mg

Mechanism of Action
MTX110 is a pan-HDAC inhibitory, which promotes activity of methyltransferase to methylate histones, leading to chromatin remodeling and increased cell cycle regulation, cellular differentiation and controlled apoptosis.

How the drug is supplied
MTX110 is supplied as a white to off-white lyophilized powder in 10mL sealed amber-glass vials which, on reconstitution, provides a clear colorless solution. Each vial contains 14mg panobinostat base.

Storage and handling
MTX110 should be stored refrigerated (2-8°C). Refer to 'Instructions for Use' for reconstitution and dilution.

Side Effects
Human Toxicity: As outlined above, 7 human subjects with DIPG have received repeated doses of MTX110 via CED which was tolerated well.

As per Table 13, to date, the only adverse event that was considered related to MTX110 was fatigue, at grade 1.

Important Treatment Considerations with MTX110

The CED direct delivery method proposed herein will in theory minimize these side effects significantly given the limited systemic exposure. This is supported by an ongoing GBM study that shows limited systemic toxicity with CED-directed brain tumor treatment (Dr. Butowski, UCSF; NCT02022644; personal communication).

Neutropenia
Neutropenic complications should be managed promptly with antibiotic support. G-CSF may be used to manage neutropenia at the investigator's discretion.

Thrombocytopenia
Thrombocytopenia complications should be managed promptly with platelet transfusions as

clinically indicated and at the investigator's discretion.

Pregnancy
The pregnancy category of MTX110 is D. Women of childbearing potential should be advised to avoid becoming pregnant while receiving treatment with MTX110. If a pregnancy is reported, the pregnancy should be followed until the outcome becomes known.

2.5 Rationale

Based upon the background information concerning intra-tumoral delivery by CED and pre-clinical and clinical studies using MTX110 in animal models, we propose a Phase 1 and early efficacy trial of CED of MTX110 in children with DIPG. There is an unmet need for effective therapy for this disease, which has no standard of care treatment other than radiotherapy. All children die from disease progression, with a median survival time of 9 months. This trial will utilize repeated intratumoral CED of MTX110 in children with newly diagnosed DIPG, following initial treatment with radiotherapy. We will assess the safety of MTX110 (up to 12 mL total volume and up to 90 μM concentration) delivered by CED. We will utilize an accelerated titration design (ATD) scheme.

2.5.1 Dose Escalation Rationale

The dose escalation design in this study begins with escalation of dose administration on consecutive days followed by total volume escalation and lastly followed by dose concentration escalation. The proposed dose *volume* escalation offers a larger volume of MTX110 to be delivered with each subsequent dose level of MTX110, up to 6 mL per treatment for 2 consecutive days of treatment per cycle (total volume 12 mL). This volume escalation provides for a larger volume of the tumor to be treated each time and ideally reaches the greatest treatment coverage of tumor.

Once the RP2D volume is reached, the *concentration* of MTX110 will then be escalated. The starting concentration of MTX110 in this study is 30 μM. This dose has been previously used in the compassionate use protocols outlined in Section 2.3 and appears to be tolerable in human subjects. Based on the volume of distribution to volume of infusion (Vd:Vi) ratio (Study MTX110-S0023T), a drug concentration of 30 μM is anticipated to result in a tissue drug concentration of 10 μM throughout the treatment volume. This concentration is higher than the *in vitro* IC50 of < 100nM, shown to have cytotoxic effects in patient-derived DIPG cell lines and also exceeds the dose of 2 μM, shown to be effective in *in vivo* studies of CED with MTX110 in murine models of DIPG and glioblastoma. However, additional pre-clinical evidence in murine models treated with CED has demonstrated that much larger concentrations of MTX110 should be tolerable and higher concentrations may offer greater therapeutic benefit. To date, MTX110 doses up to 300 μM have been delivered via CED in rodent models and no animal has demonstrated excess toxicity, including no clinically relevant central nervous toxicity and no toxicity on histopathology tissue review (Study MTX110-R0021T; Study MTX110-R0226T). In higher concentrations up to 1000 μM of MTX110 delivered the brain, post-treatment tissue demonstrated mild gliosis on histopathology (Study MTX110-

R0227T; Study MTX110-R01197T). Given the tolerability of such high concentrations in murine models up to 300 μM, we propose to dose escalate to a maximum of concentration of 90 μM in the current study, with the goal to ensure therapeutic levels of MTX110 are reached throughout the CED treatment volume.

3. STUDY DESIGN

3.1 Characteristics

This is an open-label, PNOC multi institution, Phase I/II study of MTX110 delivered by CED in patients with DIPG previously treated with external beam radiation therapy.

3.2 Number of Subjects

The number of subjects enrolled will depend upon the DLTs observed and the number of dose levels tested as the study progresses in accordance with an ATD.

Once each dose level has been assessed, a detailed review of the safety and efficacy data will be performed and discussed with the FDA and the DSMC to discuss either further dose escalation or enrollment into the phase II portion of the study. Additional subjects might be needed if dose de-escalations need to occur. At least 26 subjects will be enrolled.

Dose Administration
Initially, an accelerated titration design (ATD) will be employed. This design has the advantage of potentially reducing the number of subjects treated at sub-efficacious doses, and decreasing the accrual time for the trial. Each cohort will consist of one subject. The first subject will be started at dose level 1. A 14-day window of evaluation for toxicities is required for at least one subject at each dose level before determining the dose for the next cohort.

3.3 Inclusion Criteria

Patients must have eligibility evaluations performed within 14 days prior to registration (unless otherwise stated) and must meet all inclusion and none of the exclusion criteria. In addition, the patient must be thoroughly informed about all aspects of the study, including the study visit schedule, required evaluations and all regulatory requirements. The written informed consent must be obtained from the patient or their legal guardian prior to enrollment. The following criteria apply to all patients enrolled onto the study unless otherwise specified. Tumor biopsy is not required prior to enrollment on study, unless imaging characteristics are not consistent with DIPG diagnosis and as detailed in 3.2.1. The Inclusion Criteria will be applied **after** patients have completed radiotherapy and within the given time frame per observation as listed below. Enrollment into the trial occurs after completion of radiation therapy and if all eligibility criteria are met.

3.3.1 Patients with newly diagnosed DIPG by MRI; defined as patients with a pontine location and diffuse involvement of at least 2/3 of the pons are eligible without histologic diagnosis. For lesions with typical imaging features, biopsy is **neither encouraged nor required for eligibility**. Tumors that are biopsied will be eligible if proven to be supportive of the diagnosis of a DIPG. Consensus of diagnosis by the study team must be met.

3.3.2 Patients who have completed focal radiotherapy within 14 weeks from time of enrollment are eligible.

3.3.3 Treatment must begin at a minimum of 4 weeks after, but no later than 14 weeks after, the date of completion of focal radiotherapy.

3.3.4 Prior Chemotherapy: Patients should be at least 30 days from last chemotherapy dose prior to start of CED infusion, with exception of antibody half-lives. For antibody therapies, at least 3 half-lives of the antibody after last dose of monoclonal antibody should have passed prior to CED infusion. Patients less than 30 days from last chemotherapy dose should be discussed with the study chair(s).

3.3.5 Prior Radiation: Patients must have received prior treatment with standard focal radiotherapy as part of initial treatment for DIPG and had their last dose at least 4 weeks prior to and no later than 14 weeks from the first CED treatment. Patients beyond 14 weeks from radiation therapy but with stable disease should be discussed with the study chair.

3.3.6 Age ≥ 2 years of age to ≤ 21 years. Patients younger than 3 years of age may be enrolled on study at the discretion of the Study Chair(s) if supporting evidence that brainstem lesion represents a brainstem glioma.

3.3.7 Karnofsky ≥50 for patients >16 years of age and Lansky ≥50 for patients ≤ 16 years of age (see Appendix A). Patients who are unable to walk because of paralysis, but who are able to moblize using a wheelchair, will be considered ambulatory for the purpose of assessing the performance score.

3.3.8 Life expectancy of greater than 12 weeks measured from the date of completion of radiotherapy.

3.3.9 Corticosteroids: Patients who are receiving dexamethasone must be on a stable or decreasing dose for at least 1 week prior to registration.

3.3.10 Organ Function Requirements

3.3.10.1 Adequate Bone Marrow Function defined as:

- Peripheral absolute neutrophil count (ANC) ≥1000/mm^3 **and**
- Hemoglobin ≥8g/dl **and**
- Platelet count ≥100,000/mm^3 (transfusion independent, defined as not receiving platelet transfusions for at least 7 days prior to enrollment) **and**

- Normal coagulation defined as normal INR or per institutional guidelines.

3.3.10.2 Adequate Renal Function defined as:

- Creatinine clearance or radioisotope GFR ≥ 70mL/min/1.73 m^2 **or**
- A serum creatinine based on age/gender as follows:

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

The threshold creatinine values in this table were derived from the Schwartz formula for estimating GFR utilizing child length and stature data published by the CDC.

3.3.10.3 Adequate Liver Function defined as:

- Bilirubin (sum of conjugated + unconjugated) ≤1.5 x upper limit of normal (ULN) for age **and**
- SGPT (ALT) ≤110 U/L **and**
- Serum albumin ≥2 g/dL.

3.3.11 Adequate Neurologic Function defined as:

- Patients with seizure disorder may be enrolled if on non-enzyme inducing anticonvulsants and well controlled.

3.3.12 The effects of MTX110 on the developing human fetus are unknown. For this reason women of child-bearing potential and men must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to study entry, for the duration of study participation and 4 months after completion of MTX110 injection administration. Should a woman become pregnant or suspect she is pregnant while she or her partner is participating in this study, she should inform her treating physician immediately.

3.3.13 A legal parent/guardian or patient must be able to understand, and willing to sign, a written informed consent and assent document, as appropriate.

3.4 Exclusion Criteria

3.4.1 Patients who had clinical and/or radiographic (MRI) progression of tumor following external beam radiation therapy.

3.4.2 Patients with metastatic disease, including leptomeningeal or subarachnoid disseminated disease.

3.4.3 Patients with tumor morphology or other imaging findings that predict poor coverage of the majority of the tumor including significant tumor volume outside the pons or presence of large cysts within the tumor that would prevent adequate tumor coverage by CED. Patients with concern for adequate tumor coverage based on tumor morphology should be discussed with the study chairs.

3.4.4 Patients who are receiving any other tumor-directed therapy.

3.4.5 History of allergic reactions attributed to compounds of similar chemical or biologic composition to MTX110 or gadolinium.

3.4.6 Uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements.

3.4.7 Female patients of childbearing potential must not be pregnant or breast-feeding. Female patients of childbearing potential must have a negative serum or urine pregnancy test within 14 days of registration.

3.4.8 Patients who are unable to return for follow-up visits or obtain follow-up studies required to assess toxicity to therapy. Telemedicine visits are acceptable.

3.4.9 Patients with MRI or clinical evidence of uncontrolled tumor mass effect are excluded; the assessment of mass effect should be made by the study chairs and study neurosurgeons prior to any planned CED treatment.

3.4.10 Untreated symptomatic hydrocephalus determined by treating physician.

3.4.11 Subjects with prolonged QTc (> 450 msec) will be excluded from the study.

Important note: The eligibility criteria listed above are interpreted literally and cannot be waived.

4. REGISTRATION PROCEDURES

4.1 General Guidelines

Patients must meet all inclusion criteria and no exclusion criteria should apply. The subject or their legal parent/guardian must have signed and dated an approved, current version of all applicable consent and/or assent forms. To allow non-English speaking patients to participate in this study, bilingual health services will be provided in the appropriate language when feasible.

Registration materials will be submitted to the PNOC Operations Office as described below.

The PNOC Operations Office will forward eligibility checklist including source documentation to the Study Chair or Co-Chair as well as the Project Leader or Co-Project Leader of PNOC for review of eligibility and sign off.

Eligible patients will be registered using the UCSF OnCore® database that is used for all PNOC trials. Treatment on protocol therapy cannot be initiated prior to receiving the registration confirmation email from the PNOC Operations Office.

4.2 Reservation and Registration Process

The wait-list for study slots will be maintained by the PNOC Operations Office. Investigators can view updated information about slot availability and registration process updates on the PNOC Member's SharePoint website using their secure login and password, or by emailing a request [redacted].

To place a subject on the waitlist, investigators are to send an email [redacted] with the following information: Study number, Subject age, Consent signed date (or estimate), and Expected start date (if found eligible).

To register a subject for the study, the subject demographics (gender, ethnicity, race, month & year of birth, country code, disease site, histology, diagnosis date, name of treating physician and study specific information) along with a signed consent form and HIPAA authorization (if applicable to your institutions regulatory guidelines) will be emailed to the PNOC Operations Office [redacted]. The subject will be given the status of consented in OnCore®.

When the eligibility checklist has been completed the member institution PI and/or Coordinator will upload the completed eligibility checklist along with copies of any supporting documents into the subject's OnCore® record.

Once the necessary documents have been received and the subject eligibility has been confirmed, the PNOC Operations Office will send a confirmation e-mail to the institutional PI(s) and Research Coordinator(s) with the subject's study ID and dose information.

Detailed subject screening and registration instructions can also be found on the PNOC Member's SharePoint Wiki.

5. AGENT ADMINISTRATION

5.1 Regimen Description

Treatment will be administered to subjects after admission to an inpatient facility. Appropriate dose modifications are described in Section 5.5. No investigational or commercial agents or therapies, other than those described below, may be administered with the intent to treat the subject's malignancy.

Recommended synopsis of sequence of study events (site-specific set-up may vary with the approval of study chairs):

The procedure will take place in an operating room (OR) associated with an intraoperative MRI (iMRI) scanner, in order to facilitate confirmation of catheter placement and monitoring of infusate distribution. The configuration of the transfer system, and MR gantry allows rapid movement of an anesthetized and immobilized patient from the operating room to the MR bore, which is only separated by a door from each other. Specific surgical procedures can also be performed under sterile conditions within the actual MR room using MR-compatible equipment.

The tumor target and trajectory will be selected and planned using the BrainLab navigation system based upon an imaging study obtained prior to the date of the procedure. The experimental agent will be delivered using the BrainLab Flexible Catheter system. This system is currently being used for CED in children with DIPG at Memorial Sloan Kettering Hospital (PI: Mark Souweidane), and for a single patient CED treatment with MTX110 performed at UCSF (PI: Sabine Mueller).

1. A pre-surgical MRI (with DTI sequences) will be used in conjunction with the BrainLab iPlan software package for planning catheter placement, with a maximum of 2 catheters placed into the tumor. With each treatment, a new planning MRI will be obtained and the trajectory and location of the catheter(s) optimized. The planning MRI will be obtained within 14 days prior to the scheduled procedure.

2. Following induction of general anesthesia, and immobilization of the subject's head, a maximum of 2 catheter(s) will be placed with the assistance of MRI-guided neuronavigation using standard neurosurgical techniques. Catheter position will be confirmed by an MRI study prior to initiation of drug infusion. If the subject received a prior CED of MTX110, a new catheter location will be selected to avoid overlap of the infusate. Repeated T1-weighted sequences will be collected in real-time during the infusion to monitor infusate distribution. The maximum volume will be based on the subject's dose level.

3. We will monitor the initial infusate distribution with real-time MR imaging (approximately every 15 minutes with a duration for each sequence of about 5-8 minutes), with the final component of the highest infusion volumes being done with the patient awake so that clinical assessments can be performed, as feasible. Each patient will be monitored with repeated imaging under general anesthesia until 1.2 to 1.6 cc are infused (equals to approximately 2 to 2.5 hours assuming an average infusion rate of 10 microliters/minute). The patient will then be woken up from general anesthesia. The reminder of the volume (depending on the dose level) will be infused while the patient is awake. If needed, sedating medications will be used to avoid agitation. At the end of the infusion, the patient will be brought back to the MRI scanner for a final series of images. If there are any concerns in regards to clinical signs or vital signs, imaging might be obtained at anytime during the infusion if clinically indicated. The table below describes the approximate infusion times for each dose level assuming a maximum infusion rate of 10 microliters per minute. The estimated total anesthesia time including catheter placement and initial infusion time of 2-2.5 hours is estimated to be 5.5 hours.

Dose Level (#) and associated infusion volume	# catheters	Approximate Infusion Time with 1 Catheter (using maximum infusion rate of 10 microliter/min; adding 30 min to achieve maximum infusion rate)	Approximate Infusion Time with 2 Catheters (using maximum infusion rate of 10 microliter/min; adding 30 min to achieve maximum infusion rate)
#1: 3 ml	1	5.5 hours	2.25 hours
#2: 3 ml day 1 & 2	1	5.5 hours	2.25 hours
#3: 4 ml day 1 & 2	1	7 hours	n/a
	2	n/a	3.5 hours
#4: 5 ml day 1 & 2	1	9 hours	n/a
	2	n/a	4.5 hours
#5*: 6 ml day 1 & 2	1	12.5 hours	n/a
	2	n/a	6.5 hours

*Dose levels 6 and 7 would also take approximately the same time as Dose level 5 as the volume is the same amount

4. We anticipate that changes to the infusion parameters and catheter placement may be required based on observations from the real-time MR images. We describe two possible scenarios below, but it is also possible that unforeseen distribution variations may occur:
    a. If there is evidence of backflow of the infusate along the catheter, the infusion rate may be slowed. If backflow continues to be present on repeated imaging, the catheter(s) position will be adjusted. In general, this will involve advancement of the catheter 2-5 mm within the intra-operative MR scanner using sterile techniques. If after the adjustment there is evidence of ongoing backflow, this catheter will not be used for the infusion.
    b. If distribution from a specific catheter placement is observed outside of the target volume (based on T2-weighted images) the catheter may be advanced or withdrawn along the same trajectory (same as in (a)); or the infusion stopped and the catheter repositioned along a different trajectory. If the catheter has to be repositioned along a different trajectory, the subject will be transferred to the OR. The catheter will be removed; a new trajectory will be selected by the study team and a new catheter will be placed. In this event, we anticipate that the same entry point and burr hole can be used, but it is also possible that a new entry point may be necessary (e.g. to avoid a vascular structure). Once the new catheter is inserted, placement will be confirmed with an MR scan. Once the position is confirmed, the infusion will be re-started.
5. As described above, once a stable infusion rate is achieved, we will monitor under real time imaging for approximately 2-2.5 hours under general anesthesia for each subject in the iMRI scanner. Imaging will be performed at least every 15 minutes. This timing of

imaging will allow us to observe an infusion volume of a minimum of 1.2 – 1.6 mls with an expected distribution volume of approximately 2.4-3.5 mls using an average infusion rate of 10 microliters/min. This should be sufficient to assess adequate distribution and assess backflow.

a. Once adequate distribution is confirmed and as clinically feasible, the subject will be transferred to the adjacent OR, removed from pin fixation, awakened, and extubated. The subject will then be monitored in an area close to the MR scanner, either in the adjacent OR or post-anesthesia care unit (PACU), or transferred to ICU for completion of infusion. Once the subject is awake enough to follow commands, we will perform neurologic assessments every hour appropriate to the patient's level of consciousness and age, and continuous vital sign monitoring with blood pressure checked at least every 15 minutes. During this time, subjects may receive sedative and analgesic medications as needed. At the end of the infusion, we will obtain additional MR images to assess the final volume of distribution using the intra-operative MR scanner.

b. If subjects are assigned to a dose level that will repeat CED of MTX110 on day 2, the subject will be transferred to the intensive care unit after completion of the day 1 CED and carefully monitored overnight. The catheters will remain in place. Only subjects who recover to baseline or ≤ grade 1 toxicities will be considered for day 2 infusion (not applicable to dose level 1). If they meet criteria to start the 2nd day of CED of MTX110, the subject will be transferred back to the intra-operative MR scanner. The position of the catheter(s) will be confirmed and we will monitor under real time imaging for approximately 2-2.5 hours (same as for the Day 1 CED). Children who can tolerate imaging without anesthesia will not be sedated. Children who will not tolerate imaging without anesthesia, will be anesthetized. We will then follow the same sequence of events as outlined for Day1.

6. Once the final CED is completed, catheters will be removed following the completion of the infusion; this will either occur in the OR or in the monitoring unit close to the OR.
7. Continue to repeat cycles every 4-8 weeks, until tumor progression or the development of adverse events.
8. For each subject, each catheter infusion will be initiated at a rate of 1 microliter/min and increased by a maximum of 5 microliters/min every 15 minutes under real-time imaging. The infusion rate increase will be performed sequentially for each catheter. If there are any concerns regarding the infusate distribution, the infusion rate will be reduced. The infusion rate will increase to a maximum of 10 microliters/min for the initial CED procedure for each subject. If this infusion rate is tolerated (following the same criteria as for dose escalation in 10.2, if toxicity is thought to be related to rate of infusion), the maximum rate will increase to 15 microliters/min for the next CED procedure and only as clinically indicated. The subject's vital signs will be continuously monitored during the infusion. The rate of the infusion will be under the discretion of the study team but will never exceed the maximum allowed rate.

Assessment of safety and toxicity will include all safety evaluations over a 14-day period after

each CED.

5.2 Dose Administration

Table 5.2.1. Dose levels.

Dose level	Concentration MTX110	Total Volume (ml)	Day of therapy
1	30 μM	3	Day 1
2	30 μM	6 (3mL each day)	Day 1, 2
3	30μM	8 (4mL each day)	Day 1, 2
4	30 μM	10 (5mL each day)	Day 1, 2
5	30 μM	12 (6mL each day)	Day 1, 2
6	60 μM	12 (6mL each day)	Day 1, 2
7	90 μM	12 (6mL each day)	Day 1, 2

If dose levels 6 or 7 are not tolerated, dose de-escalation levels will be implemented as per Table 5.2.2. For example, if dose level 6 is not tolerated, dose level 5a will be tested and if dose level 7 is not tolerated, dose level 6a will be tested.

Table 5.2.2. Optional dose de-escalations pending toxicity.

Dose level	Concentration MTX110	Total Volume (ml)	Day of therapy
5[a]	45 μM	12 (6mL each day)	Day 1, 2
6[a]	75 μM	12 (6mL each day)	Day 1, 2

- MTX110 will be delivered by 1 or 2 catheters surgically placed in an intratumoral (IT) location.

- The concentration of gadoteridol (ProHance®) will be 0.5 mM for both dose levels; both agents will be combined and co-infused using the same catheters.

- Maximum infusion rate will be increased as stated above in 5.1, utilizing the same criteria for escalation as will be used for overall dose level escalation (section 10.2).

- Timing of 2nd infusion (applicable to dose level 2 and higher) will be determined pending subject's return to pre-infusion baseline neurological exam or ≤ grade 1 toxicities (with exception of toxicities anticipated to be within normal limits of post-operative recovery-listed below), but will occur no later than 48 hours after completion of infusion on day 1, i.e. if subject's neurological exam has not returned to pre-infusion baseline or has evidence of toxicities > grade 1 by 48 hours from completion of day 1 infusion, the 2nd CED procedure will be canceled.

- The following toxicities are anticipated to be within normal limits of post-operative recovery and will not preclude initiation of the 2nd (day 2) infusion:

- Grade 2 headache
- Grade 2 pain
- Grade 2 nausea

- If vomiting occurs during post-operative recovery, initiation of the 2nd (day 2) infusion may proceed only if vomiting is improving and the participant has 4-6 hours without emesis prior to dose administration.

5.2.1 Preparation of MTX110

Preparation of MTX110
Refer to 'MTX110 Instructions for Use' in SharePoint for more information. The product should be reconstituted by adding the specified amount of normal saline (see table below) via a sterile needle into the vial. The vial should be gently swirled to ensure complete reconstitution.

Table 5.2.3 Saline volumes required for reconstitution

Required dose	**Volume of normal saline**
30µM (10.49µg/mL)	See note below
40 µM (13.98 µg/ml)	10ml
60 µM (20.97 µg/ml)	6.7ml
90 µM (31.46 µg/ml)	4.5ml

To achieve 30 micromolar concentration, mix 7.5 ml of the 40 micromolar panobinostat with 2.5 ml sterile saline for injection in an empty sterile vial.

5.2.2 Addition of ProHance® to MTX110 injection

The research pharmacist will combine MTX110 solution to an empty glass vial for mixing with ProHance®. The final concentration of Prohance should be 0.5 mM. The pharmacist will confirm that this is the concentration in the final mixture.

List of Materials
1. Appropriate number of vials containing MTX110 injection
2. One vial ProHance®
3. Two 3 mL syringes with 3/4" 22 gauge needle
4. One 10 mL with 1" 20 gauge needle
5. Two 20 ml syringes with 1" 20 gauge needle
6. 2 sterile 15 or 30 mL vials
7. One 1 mL syringe with needle
8. Sterile saline for injection
9. Alcohol swabs

Preparation of ProHance® Working Solution
1. Use 3 mL syringe to draw up 1 mL of Prohance (0.5 mmol)
2. Inject 1 mL ProHance into empty 15 or 30 mL sterile multiuse vial
3. Use 20 mL syringe and draw up 9 mL sterile saline for injection
4. Inject saline into vial with ProHance

5. Swirl to mix
6. Label vial as ProHance working Solution 1:10 Dilution (50 mM)

During reconstitution, dilution, and filling, exposure to air should be minimized. Inspect for visual particles and filter if necessary. For Convection Enhanced Delivery, the product should be filled into the syringes within 24 hours of use. The combined solution will then be drawn up into a Medfusion 3500 or 4000 compatible 10 mL Luer-lock syringe and delivered to the OR.

5.2.3 Instruction for drug administration with real-time imaging

List of Required Materials

1. MTX110 injection with gadoteridol added
2. BrainLab catheter
3. Neuronavigation hardware and software
4. Medfusion 3500/4000 MRI-compatible pump
5. Medfusion 3500/4000 compatible up to 10-20 mL Luer-lock syringe
6. Biohazard/sharps container for disposal of vector

Procedure

Note: site-specific procedures that differ from the procedures below must be submitted to the study chairs for review and approval.

1) Surgical planning allowing up to 2 catheters into the tumor will be performed on a MRI scan acquired up to14 days prior to surgery.
2) The iMRI suite consists of a neurosurgical operating room separated by shielded doors from a 3T GE large bore MRI scanner (model 750W). On the day of surgery the subject will be transported to the OR and placed under general anesthesia.
    a. All members of the team who are present in the MRI suite must have documented MRI safety training. Existing standard procedures for MR safety in the iMRI suite will be followed.
    b. A member of the anesthesiology department must remain present for the entire procedure.
3) Using the pre-operative planning MR scan, and the BrainLab navigation system, BrainLab flexible catheter(s) will be inserted through burr hole(s) following pre-determined trajectories and depths, and secured to the calvarium using supplied guide ports. Prior to insertion, the catheter will be primed with MTX110/gadoteridol mixture.
4) After catheter insertion, the patient will be transported to the iMRI scanner and an MRI scan will be obtained to confirm catheter position.
5) Imaging will be performed intermittently during the initial infusion to monitor the distribution of the agents and for adjustments of catheter placement and infusion rate. The infusion volume for each catheter will be based on MR images of gadoteridol distribution acquired during the infusion procedure.
6) Once a stable infusion rate has been established for both catheters, the remaining duration of infusion has been estimated, and as clinically feasible, the subject may be transferred to the PACU or ICU to complete the infusion. Repeat MR imaging will occur at completion of the CED infusion. Subjects that cannot tolerate MRI without sedation/anesthesia will need to be sedated or undergo repeat anesthesia for the end of

infusion scan.

7) After completion of infusion at each dose level, the catheter(s) will be removed depending on the dose level. For example, the catheter will removed on day 1 at dose level 1, day 2 at dose level 2, etc.
8) It is recognized that experience is likely to result in minor refinements of this procedure. Such refinements will not require amendment of the protocol as long as they do not increase the risk for the subject.
9) Timing of follow-up infusions on day 2 will be determined pending subject's return to pre-infusion baseline neurological exam or ≤ grade 1 toxicities, but will occur no later than 48 hours after completion of infusion on day 1, i.e. if subject's neurological exam has not returned to pre-infusion baseline by 48 hours from completion of day 1 infusion, the CED procedure on day 2 will be canceled and catheters removed.

5.3 Dose Limiting Toxicity (DLT)

Toxicity will be assessed according to the NCI Common Terminology Criteria for Adverse Events Version 4.0 (CTCAE v4.0).

The Dose Limiting Toxicity Period will be the first 14 days from the start of the CED procedure.

Dose limiting toxicity (DLT) will be defined as:

- Any intolerable (as deemed by the patient or investigator) grade 2 or any grade 3 or higher neurological toxicity felt to be attributable to the CED infusion procedure or to the MTX110 injection with gadoteridol within a period of 14 days after the infusion.

- Any systemic treatment related grade 3 or higher hematologic or non-hematologic toxicity (after maximal medical management of nausea/vomiting/diarrhea) within a period of 14 days after the infusion.

If multiple toxicities are seen, the presence of a dose limiting toxicity should be based on the most severe toxicity experienced.

Dose modifications are described in Section 5.5.

5.4 General Concomitant Medication and Supportive Care Guidelines

All concurrent medical conditions and complications of the underlying malignancy will be treated at the discretion of the treating physician according to acceptable local standards of medical care. Subjects should receive analgesics, anti-emetics, antibiotics, anti-pyretics, and blood products as necessary. Although warfarin-type anticoagulant therapies are permitted, careful monitoring of coagulation parameters is imperative, in order to avoid complications of any possible drug interactions. All concomitant medications, including transfusions of blood products, will be recorded on the appropriate case report form.

Guidelines for treating certain medical conditions are discussed below; however, institutional

guidelines for the treatment of these conditions may also be used. The concomitant therapies that warrant special attention are discussed below.

Edema after CED
Standard post-operative edema immediately after CED treatment should be managed as per institutional guidelines, with anticipation that patient be tapered off dexamethasone as soon as clinically tolerated.

If edema is suspected due to worsening neurological symptoms or signs, the subject should undergo MR imaging to assess for possible edema or hemorrhage. The subject may be placed on dexamethasone, and/or the dose increased up to 0.3 mg/kg/day with a maximum of 16 mg/day or per institutional standard. Length of therapy will depend on clinical symptoms.

Antiemetic Medications
Dexamethasone and a 5-HT3 blocker (e.g., ondansetron or granisetron) may be administered to all subjects as premedications unless contraindicated for the individual subject. Anti-emetics will also be prescribed as clinically indicated during the study period.

Colony Stimulating Factors
Though unlikely to be needed, the use of granulocyte colony-stimulating factor (G-CSF) is permitted to treat subjects with neutropenia or neutropenic fever but not to allow for eligibility.

Dietary Restrictions
None.

Prohibited Medications
No drug interaction studies have been conducted specifically with MTX110. The drugs shown below are noted in the Prescribing Information for other panobinostat marketed products as interacting with panobinostat when administered orally. The relevance of these interactions to MTX110 delivered by CED is considered to be low in view of its very localized infusion directly into tumour tissue.

Because there is a potential for interaction of MTX110 injection with other concomitantly administered drugs through the cytochrome P450 system, strong inhibitors and inducers of CYP3A, CYP2D6 as well as anti-arrhythmic medicines (including amiodarone, disopyramide, procainamide, quinidine and sotalol) and drugs that are known to prolong the QT interval (including chloroquine, halofantrine, clarithromycin, methadone, moxifloxacin, bepridil and pimozide) are to be avoided the week prior to and the week following every CED infusion.The case report form must capture the concurrent use of all other drugs, over-the-counter medications, or alternative therapies. Appendix B presents guidelines for identifying medications/substances that could potentially interact with the study agent. Treatment with these agents and any other that interact with MTX110, should be avoided whenever possible and must be avoided the week prior to and following every CED infusion.

Additionally, the following therapies are not permitted during the trial:

- Other anti-neoplastic therapy, including cytotoxics, targeted agents, endocrine therapy or

other antibodies.
- Radiotherapy other than what was used as part of primary treatment.
- Any prior or concurrent investigational therapy is not permitted.

5.5 Dosing Modifications and Delays

The PNOC Study Chair or Co-Chair must be notified of any dosage modifications prior to the implementation of the dose modification. Any delays in starting the next CED treatment beyond 10 weeks must be discussed with the study chairs.

Each subject will be treated with CED of MTX110 based upon their dose level assignment. The intent is to treat every 4-8 weeks, as long as there is no evidence of tumor progression within the treated area, or intolerable toxicity.

Intra-subject modification will be allowed where: dose escalation (following dose levels in table 5.2.1) is permitted if a subject experiences a toxicity of grade 0 or 1; dose de-escalation will be followed if the toxicity is dose limiting toxicity (DLT) or worse (i.e., an intolerable grade 2 or grade 3 and above, as defined in Section 5.3); while the dose will remain unchanged if a subject experiences a moderate toxicity (i.e., moderate grade 2). At the second occurrence of any grade 2 toxicity during the first treatment cycle, or the first DLT occurrence during the first treatment cycle, the ATD will be transitioned to a standard 3+3 dose escalation design. That is, two additional subjects will be accrued at the dose level that triggered the transition. As in a standard 3+3 dose escalation design, there are three possible scenarios for this cohort of 3 subjects, if there are (is):
- two or three DLTs, the RP2D will be defined as the next lower dose level;
- zero DLTs (i.e., the transition from the ATD to the 3+3 design was triggered by the second occurrence of a grade 2 toxicity as opposed to a DLT and no DLTs were observed in the additional 2 subjects), then dose escalation will occur based on Table 5.2.1;
- one DLT, an additional 3 subjects will be entered at the same dose level.

In the last scenario with six subjects at the current dose there are two subsequent scenarios:
- only one DLT and then dose escalation can occur based on Table 5.2.1.
- more than one DLT and then the next lower dose level is the RP2D.

6. TREATMENT PLAN

Subjects with a radiographic diagnosis of DIPG are potentially eligible for this protocol. Eligibility requirements and treatment begin after the completion of standard of care radiotherapy. Each subject will sign a consent form and complete eligibility procedures prior to enrollment, and baseline procedures prior to any study procedure. Treatment consists of intratumoral treatment of MTX110 by CED. The intent is to treat every 4-8 weeks with CED infusions for up to 24 months or until tumor progression or intolerable toxicity. If subjects continue to derive clinical benefit, discussion with investigators and study sponsor will determine feasibility and clinical appropriateness to continue therapy. Treatment consists of placement of up to 2 CED catheters into the tumor. The placement of catheters at the time of surgery is planned based on an MRI obtained prior to the procedure, using a predictive

placement algorithm. The infusions are done under direct MR guidance, using co-infused gadoteridol, to assess leakage and tumor volume coverage. Toxicity is assessed during and after each CED infusion, with the protocol defined dose limiting toxicity period being 14 days after each CED procedure. Subjects may be treated at the time of progression after discussion with the Study Chair(s) and Neurosurgical Chair(s), if the progression occurs outside of the previously treated area, and if the treating physician feels the subject is experiencing clinical benefit. Baseline and on-study laboratory studies, and every 4-8 week MRIs are done to assess toxicity and tumor status, as described in the study calendar, for up to 24 months or until progression or intolerable toxicity. After completing all study treatment, final assessments will be performed, including a 30-day toxicity check. All subjects are followed for toxicity, progression-free survival and overall survival.

6.1 Study Calendar

For additional details refer to the Observations and Procedures (6.2) and Long Term Follow-up (6.3) sections.

Study Day/Visit	Eligibility (-14 days)	Baseline prior to each CED infusion (-7 days)	Day of surgery for *each* CED infusion[1]	Day after surgery for *each* CED infusion (+1 day)	Day 4 after surgery for *each* CED infusion (+/- 1 day)	Weeks 1 & 2 after *each* CED infusion	End of Treatment Visit (EOT)	30 Day Toxicity	Follow -Up
Clinical procedures									
Consent Form	X								
Study Treatment			X						
Physical + Neuro Exam	X	X		X		X	X		
Vital Signs		X	X[2]	X		X			
Medical History	X								
Toxicity assessment			X	X	X	X	X	X	X
Disease Status		X							
Performance Status	X	X		X		X	X		
Concomitant Medications	X	X		X		X	X	X	
Survival									X
ECG	X	X	X[5]	X[5]			X		
Laboratory procedures									
CBC w/ Diff	X	X				X	X		
Serum Chemistry	X	X				X	X		
Serum or urine pregnancy test[3]	X	X							
Imaging procedures									
Brain MRI/Spine MRI[6]	X	X[7]					X		
Research Studies									
Collection of circulating tumor DNA		X					X		
Quality of Life Assessments									
QoL assessment[4]		X				X[4]	X[4]		

[1]Depending on dose level, a second day of study treatment, with vital signs and toxicity assessment, will be performed.
[2] Hourly assessment of neurologic function, and continuous vital sign monitoring with blood pressure checked at least every 15 minutes.
[3]For females of childbearing age
[4]QoL assessments will take place at the following time points – baseline prior to initial surgery, every 3 months throughout

therapy, and EOT visit.The Pediatric Quality of Life (PedsQL) questionnaire will be used for participants 5-17 years old and the Functional Assessment of Cancer Therapy-Brain (FACT-Br) will be used for participants 18-21 years old.
[5] ECG is required at the end of each day of CED infusion – **not** required separately on Day After Surgery if recorded after Day 2 CED has completed.
[6]Spinal MRI only required at eligibility; as clinically indicated at other time points to coincide with Brain MRI.
[7]MRI prior to CED to be within 14 days of CED procedure

6.2 Observations and Procedures

The first CED infusion may take place no sooner than 28 days and no later than 14 weeks from the last day of radiotherapy (unless previously approved by the study chairs). Telemedicine visits are acceptable.

Eligibility (within 14 days of registration) See Section 3.3 for Inclusion/Exclusion Criteria

- Signed consent form
- Physical and neurological examination
- Brain and Spine MRI (to assess disease status and to rule out mass effect)
- Complete medical history including initial and/or other pre-radiotherapy imaging (MRIs), baseline symptoms, history of prior treatments and any residual toxicity relating to prior treatment
- Karnofsky for subjects > 16 years of age and Lansky for subjects ≤ 16 years of age (See Appendix A)
- Concomitant medication assessment
- Complete blood count (CBC) with differential and platelet count
- Blood chemistry assessment, including: Alkaline phosphatase, aspartate aminotransferase/alanine aminotransferase (ALT/AST), total bilirubin, calcium, phosphorus, blood urea nitrogen (BUN), creatinine, total protein, albumin, glucose, potassium, sodium, chloride, bicarbonate
- Serum or urine pregnancy test (for females of childbearing age)
- ECG

Baseline visit prior to first CED infusion (-7 days except for MRI and QOL). Eligibility tests done within 7 days prior may be used for Baseline Visit and do not have to be repeated.

- Physical and neurological examination
- Vital signs
- Karnofsky or Lansky Performance Status
- Disease status assessment
- Concomitant medication assessment
- Laboratory Procedures:
    - CBC with differential and platelet count
    - Blood Chemistry including: Alkaline phosphatase, aspartate aminotransferase/alanine aminotransferase (ALT/AST), total bilirubin, calcium, phosphorus, blood urea nitrogen (BUN), creatinine, total protein, albumin, glucose, potassium, sodium, chloride, bicarbonate, INR (INR as per institutional guidelines prior to surgical procedure)
    - Serum or urine pregnancy test for females of childbearing age
- ECG
- Brain MRI acquired within 14 days of surgery to be used for catheter placement planning

by iPlan® Flow with the intent to place up to 2 catheters into the tumor. MR sequences include standard T1- and T2-weighted sequences, iron-sensitive sequences, as well as DTI sequences. Spinal MRI as clinically indicated.
- Blood draw for circulating tumor DNA collection to be scheduled with standard of care laboratory evaluations.
- Quality of life assessments (-3 weeks)

Day of surgery for ***each*** CED infusion *(Note, the subject will likely be admitted to hospital for 2-5 nights depending on the dose level. Prior similar studies demonstrate that most subjects can be discharged within this time frame barring unforeseen complications)*
- Catheter(s) placed under general anesthesia by surgical image-guided neuronavigation, and position confirmed by MR imaging.
    - Correct catheter placement is defined as within the tumor.
- Continuous vital sign assessment and every 1 hour neuro check assessment when awake until completion of the procedure*
- Study treatment*
- Toxicity assessment*
- ECG*

*Based on dose level, if a second day of CED infusion is conducted, these assessments will be required following that infusion

Day after **each day of** CED infusion
- Physical and neurological examination
- Vital signs
- Karnofsky or Lansky Performance status
- Toxicity assessment
- Concomitant medications assessment

Day of discharge - *Patient should meet the following criteria for day of discharge*
- No further CED infusions required for that treatment cycle
- Return of toxicities to baseline or ≤ grade 2, with the exception of adverse events thought to be irreversible or with potential for slow recovery, as long as patient is deemed safe for discharge by primary team.

Day 4 after surgery after *each* CED infusion
- Toxicity assessment via phone or clinic visit

Weeks 1 and 2 after every CED infusion (+/- 3 days)

- Physical and neurological examination
- Vital signs
- Complete blood count (CBC) with differential and platelet count
- Serum chemistry, including: Alkaline phosphatase, aspartate aminotransferase/alanine aminotransferase (ALT/AST), total bilirubin, blood urea nitrogen (BUN), creatinine, total protein, glucose, potassium, sodium, chloride

- Karnofsky or Lansky Performance status
- Toxicity assessment
- Concomitant medications assessment

Prior to each CED infusion (2nd infusion and every infusion thereafter)

- MRI of brain, and spine if indicated, for disease assessment within 14 days prior to the next CED infusion
- Blood draw for circulating tumor DNA collection to be scheduled with standard of care laboratory evaluations
- If stable or responsive disease, then follow "Pre-treatment visit" procedures and then procedures as above.

Subjects will receive CED infusions every 4-8 weeks until disease progression or intolerable toxicity. Treatment will continue for up to 24 months or until tumor progression or intolerable toxicity. If subjects continue to derive clinical benefit, discussion with investigators and study sponsor will determine feasibility and clinical appropriateness to continue therapy.

Pre-treatment visit prior to the 2nd CED infusion and every infusion thereafter (-7 days)

- Physical and neurological examination
- Vital signs
- Karnofsky or Lansky Performance Status
- Toxicity Assessment
- Disease status assessment
- Concomitant medication assessment
- Laboratory Procedures:
    - CBC with differential and platelet count
    - Blood Chemistry including: Alkaline phosphatase, aspartate aminotransferase/alanine aminotransferase (ALT/AST), total bilirubin, calcium, phosphorus, blood urea nitrogen (BUN), creatinine, total protein, albumin, glucose, potassium, sodium, chloride, bicarbonate, INR (per standard of care)
    - Serum or urine pregnancy test for females of childbearing age
- ECG

Quality of life assessments will occur every 3 months (+/- 3 weeks) while subjects receive protocol therapy.

***Once all CED infusions are completed, and no further CED infusions are planned:**

End of Treatment Visit (+/-14 days except Quality of Life assessments which can be +/- 21 days)

- MRI of brain, and spine if indicated, (does not have to be repeated if done within 30 days of end of treatment)
- Physical and neurological examination
- Karnofsky or Lansky Performance Status
- Toxicity assessment

- Concomitant medications assessment
- Blood draw for circulating tumor DNA collection to be scheduled with standard of care laboratory evaluations
- ECG
- Quality of life assessments (unless performed within the 2 months prior)

30 Day Toxicity Check (+7 days)
These assessments may be done via phone 30 days after the last day of treatment.

- Toxicity assessment - related AEs must continue to be followed until resolution or return to baseline.
- Concomitant medication assessment

6.3 Long Term/Survival Follow-up Procedures

Subjects who are off-treatment will be followed by chart review and/or telephone contact **every two months** or until an off-study criterion is met, to collect disease and survival status information. This information will be recorded in the OnCore® eCRFs due at Follow-up.

- Subjects who are off-treatment will be followed until death. Subjects who expire without confirmation of disease status will be considered to have progressive disease at the time of death.

- Subjects will be followed to collect any adverse events that are possibly, probably or definitely related to the study drug, until resolution of those events.

6.4 Off-Treatment Criteria

Treatment may continue until one of the following criteria applies:

- Disease progression
- General or specific changes in the subject's condition render the subject unacceptable for further treatment in the judgment of the investigator
- Unacceptable adverse events
- The subject, parent or legal guardian refuses further treatment on this protocol,
- Pregnancy

The “Off Treatment Date” and reason for discontinuation must be documented by the attending investigator in the medical record and recorded in two places within OnCore®, in the ‘Follow-Up’ section of OnCore® as well as in the ‘PNOC End of Treatment eCRF.’

The “Off Arm Date” must be documented in the ‘Treatment’ section of OnCore®. The ‘Off Arm Date” should correspond with the “Off Treatment Date” and is the date the subject was discontinued from protocol treatment.

The “Last Treatment Date” is recorded in two places within OnCore®, in the ‘Follow-Up section of OnCore®’ as well as in the ‘PNOC End of Treatment eCRF’. “Last Treatment Date” is defined as the last date that the subject received protocol based therapy.

6.5 Off Study Criteria

Subjects will be considered Off Study for the following reasons:

- Subject determined to be ineligible.

- Parent, subject, or guardian withdraws consent for continued participation and all follow-up activities.
- Subject death while on study.
- Completion of protocol specific follow up period.

The date and reason for the subject coming off study must be documented in the 'Follow-Up' section of OnCore® as well as the 'PNOC End of Treatment eCRF'. No data will be collected after the "off study" date.

7. ADVERSE EVENTS

An adverse event (also known as an adverse experience) within this protocol is defined as any untoward medical occurrence associated with the study procedure (placement of catheters) or use of the drug, whether or not considered to be procedure or drug related. (Please follow directions for routine reporting provided in the Data Reporting section). Additionally, all serious adverse events must be reported in an expedited manner to allow for optimal monitoring of subject safety and care. The Expedited Reporting sections in this protocol provide guidelines for expedited reporting.

7.1 Adverse Event Characteristics

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

  Surgical adverse events that cannot be graded by CTCAE terminology (e.g., prolonged intubation) will be reviewed on a weekly basis by the Investigator and by the Data and Safety Monitoring Committee (DSMC) per their established reviewing schedule. Please see Appendix E PNOC Data Safety and Monitoring Plan for more information. All other events that can be graded by CTCAE terminology will use the CTCAE grading. This includes wound infections, hemorrhage, cerebral spinal fluid (CSF) leak, and respiratory and neurologic adverse events.

- **Attribution** of the AE:

  - Definite – The AE *is clearly related* to the study treatment including surgical procedure.
  - Probable – The AE *is likely related* to the study treatment including surgical procedure.
  - Possible – The AE *may be related* to the study treatment including surgical procedure.
  - Unlikely – The AE *is doubtfully related* to the study treatment including surgical procedure.
  - Unrelated – The AE *is clearly NOT related* to the study treatment including surgical procedure.

7.1.1 **Suspected**

A suspected adverse reaction is defined as any adverse event for which there is a reasonable possibility that the drug or study procedure caused the adverse event. For the purposes of IND safety reporting, "reasonable possibility" indicates that there is evidence to suggest a causal relationship between the drug/study procedure and the adverse event. A suspected adverse reaction implies a lesser degree of certainty about causality than an adverse reaction.

7.1.2 **Unexpected**

An adverse event or suspected adverse reaction is considered *unexpected* if it is not listed in the investigator brochure or package insert(s), or is not listed at the specificity or severity that has been observed, or, if an investigator brochure is not required or available, is not consistent with the risk information described in the general investigational plan or elsewhere in the current application.

"Unexpected," as used in this definition, also refers to adverse events or suspected adverse reactions that are mentioned in the investigator brochure as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug, but are not specifically mentioned as occurring with the particular drug under investigation.

Adverse events that would be anticipated to occur as part of the disease process are considered *unexpected* for the purposes of reporting because they would not be listed in the investigator brochure. For example, a certain number of non-acute deaths in a cancer trial would be anticipated as an outcome of the underlying disease, but such deaths would generally not be listed as a suspected adverse reaction in the investigator brochure.

Some adverse events are listed in the Investigator Brochure as occurring with the same class of drugs, or as anticipated from the pharmacological properties of the drug, even though they have not been observed with the drug under investigation. Such events would be considered *unexpected* until they have been observed with the drug under investigation. For example, although angioedema is anticipated to occur in some subjects exposed to drugs in the ACE inhibitor class and angioedema would be described in the investigator brochure as a class effect, the first case of angioedema observed with the drug under investigation should be considered *unexpected* for reporting purposes.

7.1.3 **Serious**

An adverse event or suspected adverse reaction is considered *serious* if, in the view of either the investigator or sponsor, it results in any of the following outcomes:

- Death
- Life-threatening adverse event
- Inpatient hospitalization ≥ 24 hours or prolongation of existing hospitalization by ≥ 24 hours
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life function
- Congenital anomaly/birth defect, or cancer
- Event that changes the risk/benefit ratio of the study

Important medical events that may not result in death, are life threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias

or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

7.1.4 **Life-threatening**

An adverse event or suspected adverse reaction is considered *life threatening* if, in the view of either the investigator or sponsor, its occurrence places the subject or subject at immediate risk of death. It does not include an adverse event or suspected adverse reaction that, had it occurred in a more severe form, might have caused death.

7.2 Adverse Event Monitoring

PNOC uses the web-based OnCore® Clinical Trials Management System for monitoring and recording of Adverse Events (AEs) including all adverse reactions considered "serious" (also called Serious Adverse Events, or SAEs).

All Adverse Events entered into OnCore® will be reviewed on a weekly basis by the PNOC Operations Office. The PNOC Operations Office will discuss the toxicity, grade, and relationship to study intervention for all AEs in question.

In addition, all Serious Adverse Events (SAEs) will be reviewed and monitored by the UCSF DSMC on an ongoing basis, and will be discussed at the UCSF DSMC meeting, which take place every six (6) weeks. Please see Appendix E PNOC Data Safety and Monitoring Plan for more information.

7.2.1 Surgical Adverse Event Grading

Anticipated Adverse Effects

Complications related to the surgical procedure will be documented. 'Minor' are those defined as leading to little or no effect on function, and not resulting in a significant increase in hospital stay. 'Major' complications are those resulting in permanent sequelae with functional limitations, or those leading to a significant increase in hospital stay. Surgical adverse events that cannot be graded by CTCAE terminology (e.g., prolonged intubation) will be reviewed on a weekly basis by the Investigator and by the Data and Safety Monitoring Committee (DSMC) per their established reviewing schedule. All other events that can be graded by CTCAE terminology will use the CTCAE grading. This includes wound infections, hemorrhage, cerebral spinal fluid (CSF) leak, and respiratory and neurologic adverse events.

7.3 Adverse Event Reporting

All Adverse Events (AEs) will be entered into OnCore®. Appendix D includes detailed information about PNOC reporting timelines.

The study period during which AEs and SAEs must be reported begins after informed consent is obtained and ends 30 days following the last administration of study treatment. After this period, only SAEs that are attributable to study treatment should be reported. Subjects removed from therapy for unacceptable adverse event(s) will be followed until adverse event(s) resolves or returns to baseline status.

The PNOC Investigator will assign attribution of the possible association of the event with use of the study therapy, and this information will be entered into OnCore®. The Investigator must also comply with all reporting requirements to their institutional Data and Safety Monitoring Committee (DSMC) and Institutional Review Board (IRB).

7.4 Serious Adverse Events and Expedited Reporting

All Adverse Events which meet the definition of 'Serious' as well as other medically significant events described below require expedited reporting to PNOC. Below are instructions for recording and reporting of these events. Please contact the PNOC Operations Office [redacted] with any questions regarding expedited reporting requirements for this study. See 7.1.3 for the definition of an SAE.

All SAEs (see above definition) on any PNOC trial, regardless of relationship, must be reported to PNOC via OnCore and Email within one business day of first PI awareness, even if the SAE is ongoing. The SAE must be followed until resolution:

- **OnCore:** All SAEs must be entered into the Subject Console in OnCore (https://oncore.ucsf.edu/ > Subject Console > SAE Tab on left). The OnCore SAE record should be updated immediately as new information becomes available until the SAE is resolved. (Toxicity segment MUST be completed. Don't forget to click "Add" button.) Please refer to the "PNOC OnCore SAE Entry Guide: Field by Field" for more information.
- **Email:** Please also email [redacted] with, at minimum, the following information:

  In the subject line: "SAE: Subject PNOC ID" (e.g. "SAE: PNOCxxx-1")

  In the body of the email: Subject PNOC ID and the OnCore assigned SAE number
- **Site IRB:** Each PNOC site is also responsible for following their own IRB guidelines for reporting SAEs.

SAE Data Entry in AE CRF:

All SAEs must also be entered into the AE CRF for that Cycle (Subject Console > Forms by Status/Forms by Visit). This entry must take place within 10 days of the last day of the Cycle in which the SAE occurred, or as soon as possible in the case of an SAE that was discovered late. Please reference the "PNOC OnCore SAE Reporting and Entry" in SharePoint for more information.

SAE Deviations:

If the protocol procedures around SAEs are not followed (e.g. reporting timelines or dose modifications), a Deviation may also need to be entered in OnCore (Subject Console > Deviation Tab on left). Please reference the "PNOC Deviation Reporting Guidelines" in SharePoint for more information.

7.4.1 Medically Significant Events

Email notification to PNOC Operations Office [redacted] **within one business day** of first PI awareness:

- Reports of pregnancy exposure (pregnancy encompasses the entire cycle of pregnancy and delivery, perinatal and neonatal outcomes, even if there were no abnormal findings; both maternal and paternal exposure is collected)
- Reports of lactation exposure

- Overdose (with or without an SAE)
- Abuse (use for non-clinical reasons with or without an SAE)
- Inadvertent or accidental exposure

7.4.2 PNOC Reporting to the UCSF Data and Safety Monitoring Committee

If a death occurs during the treatment phase of the study, or within 30 days after the last administration of the study drug(s), and is determined to be related either to the investigational drug or to any research related procedure, the Study Chair and the PNOC Operations Office must be notified by the member institution **within 1 business day**. The Study Chair or the PNOC Operations Office must then notify the UCSF DSMC Chair, or qualified alternate, within 1 business day of this notification. The contact may be by phone or e-mail. Each participating site will follow their institutional reporting guidelines to institutional DSMC.

7.4.3 PNOC Reporting to UCSF Institutional Review Board (IRB)

The PNOC Operations Office must report events meeting the UCSF IRB definition of "Unanticipated Problem" (UP) **within 10 business days** of awareness of the event.

Each participating site will follow their institutional reporting guidelines to the IRB.

7.4.4 PNOC Reporting to the Food and Drug Administration (FDA)

If the study is being conducted under an IND, the PNOC Operations Office is responsible for determining whether or not the suspected adverse reaction meets the criteria for expedited reporting in accordance with Federal Regulations (21 CFR §312.32).

The PNOC Operations Office must report in an IND safety report any suspected adverse reaction that is both serious and unexpected. The Sponsor-Investigator (PNOC) needs to ensure that the event meets all three definitions:

- Suspected adverse reaction
- Unexpected
- Serious

If the adverse event does not meet all three of the definitions, it should not be submitted as an expedited IND safety report.

The timeline for submitting an IND safety report to FDA is no later than **15 calendar days** after the PNOC Operations Office determines that the suspected adverse reaction qualifies for reporting (21 CFR 312.32(c)(1)).

Any unexpected fatal or life-threatening suspected adverse reaction will be reported to FDA no later than **7 calendar days** after PNOC initial receipt of the information (21 CFR 312.32(c)(2)).

Any relevant additional information that pertains to a previously submitted IND safety report will be submitted to FDA as a Follow-up IND Safety Report without delay, as

soon as the information is available (21 CFR 312.32(d)(2)).

7.4.5 PNOC Reporting to Midatech Pharma (supplier of MTX110 injection)

The Sponsor-Investigator (PNOC) is responsible for reporting of serious adverse events to Midatech Pharma. SAEs should be reported to the Chief Medical Officer at Midatech Pharma [redacted] within one business day using the same criteria described in Section 7.4.3 PNOC Reporting to the UCSF Data and Safety Monitoring Committee. Additionally, copies of all PNOC safety reports to the FDA and any related correspondence should be simultaneously copied to Midatech.

Periodic safety reports with tabulation of all AEs and SAEs should be provided to Midatech every 3 months during the study.

7.5 Secondary Malignancy

A *secondary malignancy* is a cancer caused by treatment for a previous malignancy (*e.g.*, treatment with investigational agent/intervention, radiation or chemotherapy). A secondary malignancy is not considered a metastasis of the initial neoplasm.

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

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

8. PHARMACEUTICAL INFORMATION

8.1 Study Agent

8.1.1 *MTX110 injection*

Product description: MTX110 is the study agent supplied by Midatech Pharma. The drug is a formulation of liquid panobinostat. This agent is not FDA approved for the treatment of primary CNS brain tumors or brainstem glioma (DIPG).

How the drug is supplied: The product is presented 10 ml amber glass vials at a concentration of 40μM (13.98μg/ml).

Storage and handling: The product is shipped under refrigerated conditions (2°C to 8°C). On receipt at the pharmacy, the product should be removed for the shipping package and stored at refrigerated conditions (2°C to 8°C) prior to reconstitution for use.

Ordering: Pharmacy will order directly from Midatech Pharma.

Stability: When stored in vials at 5°C the product remains stable for at least 42 days. Once reconstituted the product should be used within 24 hours.

Route of administration: Intratumoral infusion via convection enhanced delivery (CED). See Section 5.0 for specific details.

Accountability: The investigator, or a responsible party designated by the investigator, will be responsible for drug accountability and this will be managed per each PNOC institution guidelines.

9. EVALUATION CRITERIA

Although response is not the primary endpoint of this trial, subjects with measurable disease will be assessed by standard imaging criteria.

9.1 Response Criteria

9.1.1 Definitions

Evaluable for toxicity. All subjects will be evaluable for toxicity from the time of their first treatment with MTX110 injection.

Evaluable for objective response. Only those subjects who have measurable disease present at baseline, have received at least one cycle of therapy, and have had their disease re-evaluated will be considered evaluable for response. These subjects will have their response classified according to the definitions stated below. (Note: Subjects who exhibit objective disease progression prior to the end of cycle 1 will also be considered evaluable.)

Evaluable Non-Target Disease Response. Subjects who have lesions present at baseline that are evaluable but do not meet the definitions of measurable disease, have received at least one cycle of therapy, and have had their disease re-evaluated will be considered evaluable for non-target disease. The response assessment is based on the presence, absence, or unequivocal progression of the lesions.

9.1.2 Disease Parameters

Measurable disease

Measurable disease is defined as lesions that can be accurately measured in two dimensions (longest diameter to be recorded) with a minimum size of no less than double the slice thickness.

All tumor measurements will be recorded in millimeters or decimal fractions of centimeters. Previously irradiated lesions are considered non-measurable except in cases of documented progression of the lesion since the completion of radiation therapy.

All measurements will be taken using a ruler or calipers. All baseline evaluations will be performed as closely as possible to the beginning of treatment and never more than 30 days before the beginning of the treatment.

The same method of assessment and the same technique will be used to characterize each identified and reported lesion at baseline and during follow-up.

Non-measurable disease

Non-measurable disease is all other lesions (or sites of disease), including small lesions (< double the slice thickness).

Target and Non-target lesion

Tumor dimensions are determined by measurement of the longest tumor dimension and its perpendicular for each target lesion. For most CNS tumors, only one lesion/mass is present and therefore is considered a "target" for measurement/follow up to assess for tumor progression/response. If multiple measurable lesions are present, up to 3 can be selected as "target" lesions. Target lesions should be selected on the basis of size and suitability for accurate repeated measurements. All other lesions will be followed as non-target lesions (including CSF positive for tumor cells). The lower size limit of the target lesion(s) should be at least twice the thickness of the slices showing the tumor to decrease the partial volume effect.

Tumor Measurements

Regarding MRI imaging, the sequence that best highlights the tumor (T1 enhanced or T2 weighted or FLAIR images) will be chosen to determine response criteria. The same sequence should be used for serial measurements. Response determination will be based on a comparison of an area [**W** (longest diameter of the target lesion) **x T** (transverse measurement, perpendicular to W)] between the baseline assessment and the study date designated in the follow-up Report Form. Reports for the follow-up exams should reiterate the measurements obtained at baseline for each target lesion. Nontarget lesions or newly occurring lesions should also be enumerated in these reports, and changes in non-target lesions should be described.

1. For MRI imaging, the longest diameter can be measured from the axial plane or the plane in which the tumor is best seen or measured. The longest measurement of the tumor is referred to as the width (W).
2. The perpendicular measurements should be determined - transverse (T) measurement, perpendicular to the width in the selected plane.
3. The cystic or necrotic components of a tumor are not considered in tumor measurements. Therefore only the solid component of cystic/necrotic tumors should be measured. If cysts/necrosis composes the majority of the lesion, the lesion may not be "measurable".

Options:

- If the cyst/necrosis is eccentric, the W and T of the solid portion should be measured, the cyst/necrosis will be excluded from measurement
- If the cyst/necrosis is central but represents a small portion of the tumor (< 25%), disregard and measure the whole lesion
- If the cyst/necrosis is central but represents a large portion of the tumor, identify a solid aspect of the mass that can be reproducibly measured
- Leptomeningeal tumor spread is usually not a target lesion, and usually cannot be measured accurately. Presence and location of leptomeningeal tumor spread should be noted and change in extent/thickness assessed on follow up studies.

Overall Response Assessment:

The overall response assessment takes into account response in both the target and non-target lesion, and the appearance of new lesions, where applicable, according to the criteria described in the table below. The best overall response is the best response recorded from the start of the treatment until disease progression/recurrence (taking as reference for progressive disease the smallest measurements recorded since the treatment started). The subject's best response assignment will depend on the achievement of both measurement and confirmation criteria.

Target Lesion(s)	Non Target Lesion (s)	New Lesions	Overall Response	Best Response for this Category Also Requires
CR	CR	No	CR	> 8 weeks confirmation
CR	IR/SD	No	PR	> 8 weeks confirmation
PR	CR/IR/SD	No	PR	
SD	CR/IR/SD	No	SD	documented at least > 4 weeks from baseline
PD	Any	Yes or No	PD	
Any	PD	Yes or No	PD	
Any	Any	Yes	PD	

CR – Complete Response; PR – Partial Response; SD – Stable Disease; PD – Progressive Disease; IR – Incomplete Response

Response Criteria for Target/Non-Target Lesions:

Response criteria are assessed in 2 dimensions – the product of W x T.
To assess response/progression, the ratio is calculated: **W x T (current scan) divided by W x T (reference scan).** Development of new disease or progression in any established lesions is considered progressive disease, regardless of response in other lesions – e.g. when multiple lesions show opposite responses, the progressive disease takes precedence.

For purposes of this study, response criteria for target lesions are:

Complete Response (CR): Complete disappearance of all known disease for at least 8 weeks. Complete response is dated from the time all lesions have disappeared on a stable or decreasing dose of corticosteroids.
Partial response (PR): A reduction of at least 50% in the size of all measurable tumors

as quantitated by the sum of the products of the largest diameters of measurable lesions and maintained for at least 8 weeks on a stable or decreasing dose of corticosteroids. Partial response is dated from the time of first observation. In addition, there can be no appearance of new lesions or progression of any lesion.
Stable Disease (SD): A decrease of <50% or an increase of <25% in the sum of the products of the largest diameters of measurable lesions and no evidence of new lesions for at least 4 weeks on a stable or decreasing dose of corticosteroids.
Progressive Disease (PD): ≥ 25% increase in the sum of the products of the largest diameters of the measurable lesions or the appearance of one or more new lesions.

9.1.3 Duration of Response

Duration of overall response: The duration of overall response is measured from the time measurement criteria are met for CR or PR (whichever is first recorded) until the first date that recurrent or progressive disease is objectively documented (taking as reference for progressive disease the smallest measurements recorded since the treatment started).

The duration of overall CR is measured from the time measurement criteria are first met for CR until the first date that progressive disease is objectively documented.

Duration of stable disease: Stable disease is measured from the start of the CED infusion treatment until the criteria for progression are met, taking as reference the smallest measurements recorded since the treatment started, including the baseline measurements.

9.1.4 Progression-Free Survival

PFS is defined as the duration of time from diagnosis to time of progression or death, whichever occurs first.

9.1.5 Overall survival

OS is defined as the duration of time from diagnosis to time of death.

9.2 Imaging Analyses

At the end of the study, all images will be evaluated by central review. Please see Appendix H for details. All imaging from initial diagnosis and/or prior to radiotherapy, as well as available imaging collected on study and/or in follow up must be submitted for central review and study analyses. Examples of imaging analyses may include, but are not limited to, descriptive correlation of imaging characteristics and clinical outcomes, and imaging correlates with treatment response or resistance.

10. STATISTICAL CONSIDERATIONS

10.1 Study Design/Endpoints

This is an open-label, prospective, multi institutional safety and early efficacy protocol. All subjects will be treated at a PNOC Institution or participating institution for this study. The primary endpoint is safety; the secondary endpoint is a preliminary estimate of efficacy.

Primary Endpoint:

- Safety and toxicity measurements of repeated CED administration of MTX110 following standard of care focal radiotherapy using CTCAE version 4.0 in newly diagnosed subjects with DIPG.

Secondary Endpoint:

- OS at 12 months (OS12) in newly diagnosed subjects with DIPG.

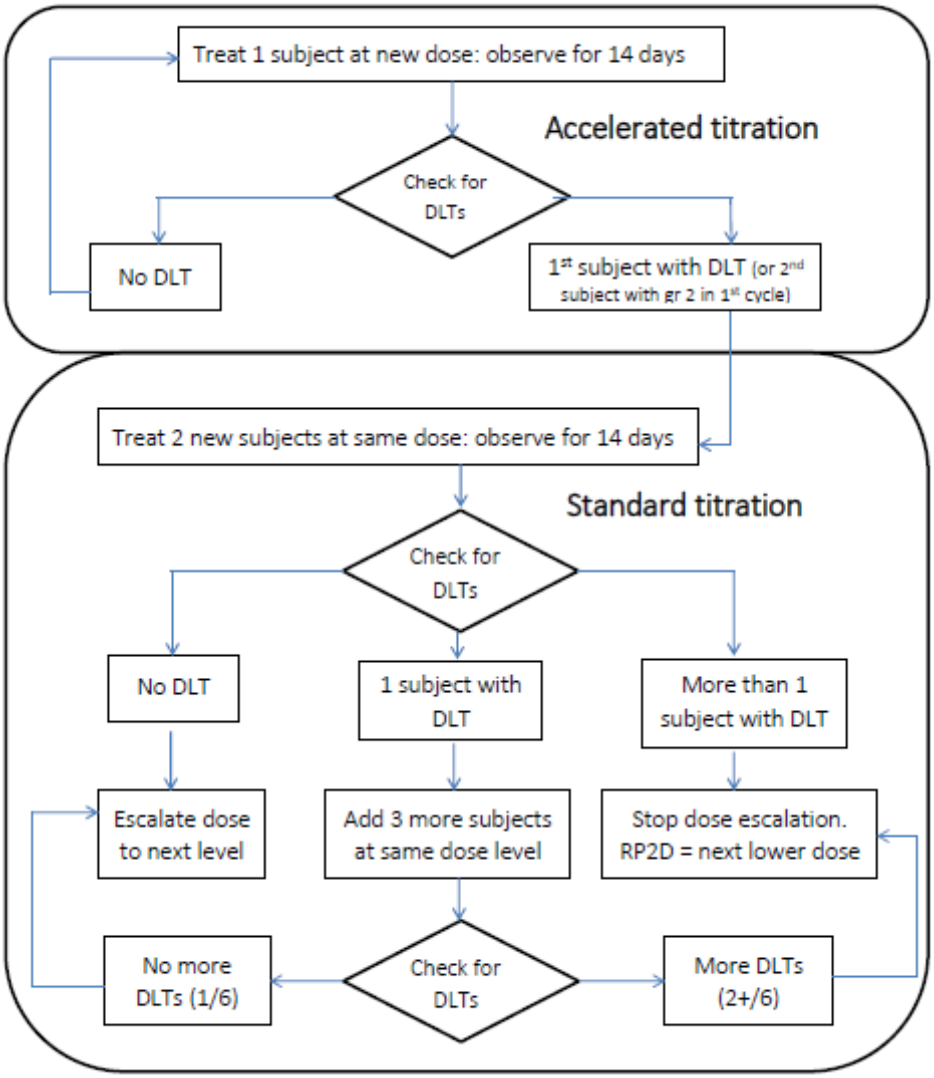
Exploratory Endpoints:

- Assessment of observed distribution of gadoteridol compared to pre-treatment modeling of the drug distribution utilizing predictive imaging software.
- The Quality of Life exploratory endpoint associated with this study will be descriptive and will be limited to frequency tables and summary statistics.
- To perform central review of imaging to explore MR qualitative and quantitative measures as markers of disease response and/or progression and treatment effect in comparison to institutional evaluation of disease response and/or progression.

10.2 Dose Escalation

Initially an accelerated titration design (ATD; diagram 1) will be employed. This design has the advantage of potentially reducing the number of subjects treated at sub-efficacious doses, and decreasing the accrual time for the trial. Each cohort will consist of one subject. The first subject will be started at dose level 1. A 14-day window of evaluation for toxicities is required for at least one subject at each dose level before determining the dose for the next cohort.

Diagram 1. Accelerated trial design.



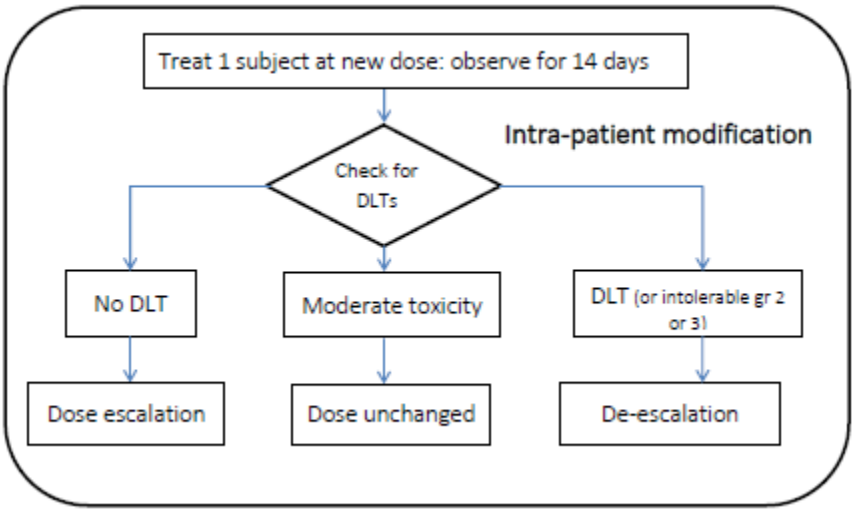
Intra-patient dose modification (Diagram 2) will be allowed as follows: dose escalation (following the dose levels in Table 5.2.1) is permitted if a subject experiences a toxicity of grade 0 or 1; dose de-escalation will be followed if the toxicity is dose limiting toxicity (DLT) or worse (i.e., an intolerable grade 2 or grade 3 and above, as defined in Section 5.3); while the dose will remain unchanged if a subject experiences a moderate toxicity (i.e., moderate grade 2). At the second occurrence of any grade 2 toxicity during the first treatment cycle, or the first DLT occurrence during the first treatment cycle, the ATD will be transitioned to a standard 3+3 dose escalation design. That is, two additional subjects will be accrued at the dose level that triggered the transition. As in a standard 3+3 dose escalation design, there are three possible scenarios for this cohort of 3 subjects, if there are (is):

- two or three DLTs, the RP2D will be defined as the next lower dose level;
- zero DLTs (i.e., the transition from the ATD to the 3+3 design was triggered by the second occurrence of a grade 2 toxicity as opposed to a DLT and no DLTs were observed in the additional 2 subjects), the dose escalation will occur based on Table 5.2.1;
- one DLT, an additional 3 subjects will be entered at the same dose level.

In the last scenario with six subjects at the current dose there are two subsequent scenarios:

- only one DLT and then dose escalation can occur based on Table 5.2.1.
- more than one DLT and then the next lower dose level is the RP2D.

Diagram 2. Intra-patient dose escalation.



10.3 Sample Size and Accrual

The number of subjects enrolled in the safety study will depend upon the DLTs observed and the number of dose levels tested as the study progresses in accordance with an ATD followed by a 3+3 design. The ATD is expected to enroll 1 to 7 subjects with a minimum of 5 subjects in the 3+3 design. Additional subjects may be enrolled depending on the dose de-escalations, expansion of dose exploration, or exploration of intermediate doses that occur. Once these dose levels have been assessed, a detailed review of the safety and efficacy data will be performed and discussed with the FDA and the DSMC to discuss either further dose escalation or enrollment into the efficacy study.

Secondary Objective: The most recent Children's Oncology Group study that treated children newly diagnosed with DIPG with a combination of radiation therapy and temozolomide resulted in an OS12 rate of 40% (SD ± 6.5%). A sample size of 19 patients achieves 80% power to detect a difference of 30% using a one sided exact binominal test. The target significance level is 5% and the actual significance level is 3.5% with this test. If 12 or more patients are alive at 12 months, the null hypothesis that OS12 is 40% will be rejected.

Stopping Rules:
Both the ATD and 3+3 have inherent stopping rules; however, it should be noted that this study will be stopped for further review by the DSMC and study team as well as FDA by:

- The occurrence of 1 or more irreversible neurological deficits that are grade 3 or higher and definitely related to the procedure based on evidence on MRI such as intraparenchymal hemorrhage, worsening edema or necrosis.

- If more than 20% of subjects develop significant, irreversible deterioration during the infusion.
- If more than 20% of subjects do not get study drug infused due to surgical complications during the catheter placement.

10.4 Stratification Factors

There are no stratification factors.

10.5 Analysis of Endpoints

Intent-to-Treat Population (ITT): The ITT population will include all subjects who are enrolled in the study. The ITT population will be the primary population for evaluating efficacy and subject characteristics.

As-Treated Population (AT): The AT population will include all subjects who receive at least 1 dose of study drug. The AT population will be the primary population for evaluating safety.

Analysis of Primary Endpoint

- Safety of repeated CED of MTX110 following standard of care focal radiotherapy will be assessed by monitoring for adverse events, scheduled laboratory assessments, vital sign measurements, and physical examinations for subjects who receive the drug. The severity of toxicities will be graded according to the NCI CTCAE v4.0. Adverse events and clinically significant laboratory abnormalities (meeting Grade 3, 4, or 5 criteria according to CTCAE) will be summarized by maximum intensity and relationship to study drug(s). Grade 1 and 2 adverse events will be summarized if related to study therapy. Descriptive statistics will be utilized to display the data on toxicity.

Analysis of Secondary Endpoint

- We will assess the preliminary efficacy using overall survival at 12 months (OS12) in 19 evaluable DIPG patients treated within the expansion cohort which will include subjects treated as part of the dose escalation portion of the trial on the recommended phase 2 dose (RP2D). Analyses will be performed after all enrolled subjects have completed 12 months, or whenever the status of all subjects has been established, whichever comes first. The primary analysis will be based on the Kaplan-Meier method. Kaplan-Meier estimates and the associated 95% CIs will be calculated for OS12. As described above, if 12 or more of the 19 patients are alive at 12 months, the null hypothesis that OS12 is 40% will be rejected.

Analysis of Exploratory endpoint

The exploratory aims associated with this study will be descriptive and will be limited to frequency tables and summary statistics.

11. DATA REPORTING / REGULATORY REQUIREMENTS

11.1 Data Reporting

11.1.1 Method

The Principal Investigator and/or his/her designee will prepare and maintain adequate and accurate participant case histories with observations and data pertinent to the study. Study specific Case Report Forms (CRFs) will document safety and treatment outcomes for safety monitoring and data analysis. All study data will be entered into OnCore® via standardized CRFs in accordance with the CTMS study calendar, using single data entry with a secure access account. The Clinical Research Coordinator (CRC) will complete the CRFs as soon as possible upon completion of the study visit; the Investigator will review and approve the completed CRFs.

The information collected on CRFs shall be identical to that appearing in original source documents. Source documents will be found in the subject's medical records maintained at each PNOC site. For participating sites, source documents will be maintained per institutional guidelines. All source documentation should be kept in separate research folders for each subject.

In accordance with federal regulations, the Investigator is responsible for the accuracy and authenticity of all clinical and laboratory data entered onto CRFs. The PI will approve all completed CRFs to attest that the information contained on the CRFs is true and accurate.

All source documentation and CTMS data will be available for review/monitoring by the UCSF DSMC and regulatory agencies.

The Principal Investigator will be responsible for ensuring the accurate capture of study data. At study completion, when the CRFs have been declared to be complete and accurate, the database will be locked. Any changes to the data entered into the CRFs after that time can only be made by joint written agreement among the Study Chair, the Trial Statistician, and the PNOC Project Leader.

11.1.2 Responsibility for Data Submission

Please refer to Appendix C for data submission timelines.

11.2 PNOC Oversight and Monitoring Plan

Given the complexity and technical requirements of this study, PNOC has determined that this should initially be a limited institutional trial (n=2). All subjects will be treated at the University of California San Francisco (UCSF) or the Memorial Sloan Kettering Cancer Center, where study Surgical Chair Dr. Mark Souweidane has experience in CED technique. Additional PNOC institutions will be allowed to open the study if the surgical PI from that site is able to observe in person a CED procedure at either UCSF or Memorial Sloan Kettering to learn the particularities of the study. The UCSF Helen Diller Family Comprehensive Cancer Center Data Safety Monitoring Committee (DSMC) will be the main monitoring entity for this study. The UCSF DSMC will work together with UCSF to monitor the study in accordance with the UCSF NCI approved Data Safety and Monitoring Plan (DSMP). The DSMC will routinely review all adverse events and suspected adverse reactions considered "serious." The UCSF DSMC will audit study-related activities to ensure that the study is conducted in accordance with the

protocol, local UCSF standard operating procedures, FDA regulations, and Good Clinical Practice (GCP). Significant results of the DSMC audit will be communicated to the IRB and the appropriate regulatory authorities at the time of continuing review, or in an expedited fashion, as applicable. Please see Appendix E PNOC Data Safety and Monitoring Plan for more information.

11.3 Multicenter Communication

The PNOC Operations Office provides administration, data management, and organizational support for the participating sites in the conduct of the clinical trial. The PNOC Operations Office will coordinate, at minimum, quarterly conference calls with the PNOC member institutions to discuss risk assessment. The following items will be discussed, as appropriate:

- Enrollment information
- Cohort updates (i.e. DLTs)
- Adverse events (i.e. new adverse events and updates on unresolved adverse events and new safety information)
- Protocol violations
- Other issues affecting the conduct of the study

11.4 Record Keeping and Record Retention

The Principal Investigator for each PNOC institution is required to maintain adequate records of the disposition of the drug, including dates, quantity, and use by subjects, as well as written records of the disposition of the drug when the study ends per institutional guidelines.

The site Principal Investigator is required to prepare and maintain adequate and accurate case histories that record all observations and other data pertinent to the investigation on each individual administered the investigational drug or employed as a control in the investigation. Case histories include the case report forms and supporting data including, for example, signed and dated consent forms and medical records including, for example, progress notes of the physician, the individual's hospital chart(s), and the nurses' notes. The case history for each individual shall document that informed consent was obtained prior to participation in the study.

Study documentation includes all CRFs, data correction forms or queries, source documents, Sponsor-Investigator correspondence, monitoring logs/letters, and regulatory documents (e.g., protocol and amendments, IRB correspondence and approval, signed subject consent forms).

Source documents include all recordings of observations or notations of clinical activities and all reports and records necessary for the evaluation and reconstruction of the clinical research study.

In accordance with FDA regulations, the investigator shall retain records for a period of 2 years following the date a marketing application is approved for the drug for the indication for which it is being investigated; or, if no application is to be filed or if the application is not approved for such indication, until 2 years after the investigation is discontinued.

11.5 Coordinating Center Documentation of Distribution

It is the responsibility of the PNOC Operations Office to maintain adequate files documenting the distribution of study documents as well as their receipt (when possible). The HDFCCC

recommends that the PNOC Operations Office maintain a correspondence file and log for each segment of distribution (e.g., FDA, drug manufacturer, participating sites, etc.).

Correspondence file: should contain copies (paper or electronic) of all protocol versions, cover letters, amendment outlines (summary of changes), etc., along with distribution documentation and (when available) documentation of receipt.

Correspondence log: should be a brief list of all documents distributed including the date sent, recipient(s), and (if available) a tracking number and date received.

At a minimum, the PNOC Operations Office must keep documentation of when and to whom the protocol, its updates and safety information are distributed.

11.6 Regulatory Documentation

Prior to implementing the protocol at each PNOC institution, the protocol, informed consent form, HIPAA authorization and any other information pertaining to participants must be first approved by the UCSF Institutional Review Board (IRB) and by the PNOC Operations Office. Prior to implementing this protocol at the participating sites, approval for the UCSF IRB approved protocol must be obtained from the participating site's IRB.

Appendix C lists the documents which must be provided to PNOC Operations Office before the participating site can be initiated and begin enrolling participants.

Upon receipt of the required documents, PNOC Operations Office will formally contact the site and grant permission to proceed with enrollment.

REFERENCES

1. Bobo, R.H., et al., *Convection-enhanced delivery of macromolecules in the brain.* Proc Natl Acad Sci U S A, 1994. **91**(6): p. 2076-80.
2. Morrison, P.F., et al., *High-flow microinfusion: tissue penetration and pharmacodynamics.* Am J Physiol, 1994. **266**(1 Pt 2): p. R292-305.
3. Szerlip, N.J., et al., *Real-time imaging of convection-enhanced delivery of viruses and virus-sized particles.* J Neurosurg, 2007. **107**(3): p. 560-7.
4. Noble, C.O., et al., *Novel nanoliposomal CPT-11 infused by convection-enhanced delivery in intracranial tumors: pharmacology and efficacy.* Cancer Res, 2006. **66**(5): p. 2801-6.
5. Hadaczek, P., et al., *The "perivascular pump" driven by arterial pulsation is a powerful mechanism for the distribution of therapeutic molecules within the brain.* Mol Ther, 2006. **14**(1): p. 69-78.
6. Hamilton, J.F., et al., *Heparin coinfusion during convection-enhanced delivery (CED) increases the distribution of the glial-derived neurotrophic factor (GDNF) ligand family in rat striatum and enhances the pharmacological activity of neurturin.* Exp Neurol, 2001. **168**(1): p. 155-61.
7. Neeves, K.B., et al., *Dilation and degradation of the brain extracellular matrix enhances penetration of infused polymer nanoparticles.* Brain Res, 2007. **1180**: p. 121-32.
8. Nguyen, J.B., et al., *Convection-enhanced delivery of AAV-2 combined with heparin increases TK gene transfer in the rat brain.* Neuroreport, 2001. **12**(9): p. 1961-4.
9. Morrison, P.F., et al., *Focal delivery during direct infusion to brain: role of flow rate, catheter diameter, and tissue mechanics.* Am J Physiol, 1999. **277**(4 Pt 2): p. R1218-29.
10. Chen, M.Y., et al., *Variables affecting convection-enhanced delivery to the striatum: a systematic examination of rate of infusion, cannula size, infusate concentration, and tissue-cannula sealing time.* J Neurosurg, 1999. **90**(2): p. 315-20.
11. Krauze, M.T., et al., *Reflux-free cannula for convection-enhanced high-speed delivery of therapeutic agents.* J Neurosurg, 2005. **103**(5): p. 923-9.
12. Fiandaca, M.S., et al., *Real-time MR imaging of adeno-associated viral vector delivery to the primate brain.* Neuroimage, 2009. **47 Suppl 2**: p. T27-35.
13. Nguyen, T.T., et al., *Convective distribution of macromolecules in the primate brain demonstrated using computerized tomography and magnetic resonance imaging.* J Neurosurg, 2003. **98**(3): p. 584-90.
14. Krauze, M.T., et al., *Real-time visualization and characterization of liposomal delivery into the monkey brain by magnetic resonance imaging.* Brain Res Brain Res Protoc, 2005. **16**(1-3): p. 20-6.
15. Murad, G.J., et al., *Real-time, image-guided, convection-enhanced delivery of interleukin 13 bound to pseudomonas exotoxin.* Clin Cancer Res, 2006. **12**(10): p. 3145-51.
16. Lonser, R.R., et al., *Image-guided, direct convective delivery of glucocerebrosidase for neuronopathic Gaucher disease.* Neurology, 2007. **68**(4): p. 254-61.
17. Fiandaca, M.S., et al., *Image-guided convection-enhanced delivery platform in the treatment of neurological diseases.* Neurotherapeutics, 2008. **5**(1): p. 123-7.
18. Varenika, V., et al., *Detection of infusate leakage in the brain using real-time imaging of convection-enhanced delivery.* J Neurosurg, 2008. **109**(5): p. 874-80.

19. Gill, S.S., et al., *Direct brain infusion of glial cell line-derived neurotrophic factor in Parkinson disease.* Nat Med, 2003. **9**(5): p. 589-95.
20. Marks, W.J., Jr., et al., *Gene delivery of AAV2-neurturin for Parkinson's disease: a double-blind, randomised, controlled trial.* Lancet Neurol, 2010. **9**(12): p. 1164-72.
21. Laske, D.W., R.J. Youle, and E.H. Oldfield, *Tumor regression with regional distribution of the targeted toxin TF-CRM107 in patients with malignant brain tumors.* Nat Med, 1997. **3**(12): p. 1362-8.
22. Sampson, J.H., et al., *Progress report of a Phase I study of the intracerebral microinfusion of a recombinant chimeric protein composed of transforming growth factor (TGF)-alpha and a mutated form of the Pseudomonas exotoxin termed PE-38 (TP-38) for the treatment of malignant brain tumors.* J Neurooncol, 2003. **65**(1): p. 27-35.
23. Weber, F.W., et al., *Local convection enhanced delivery of IL4-Pseudomonas exotoxin (NBI-3001) for treatment of patients with recurrent malignant glioma.* Acta Neurochir Suppl, 2003. **88**: p. 93-103.
24. Sampson, J.H., et al., *Poor drug distribution as a possible explanation for the results of the PRECISE trial.* J Neurosurg, 2010. **113**(2): p. 301-9.
25. Weber, F., et al., *Safety, tolerability, and tumor response of IL4-Pseudomonas exotoxin (NBI-3001) in patients with recurrent malignant glioma.* J Neurooncol, 2003. **64**(1-2): p. 125-37.
26. Sampson, J.H., et al., *Intracerebral infusion of an EGFR-targeted toxin in recurrent malignant brain tumors.* Neuro Oncol, 2008. **10**(3): p. 320-9.
27. Kunwar, S., et al., *Direct intracerebral delivery of cintredekin besudotox (IL13-PE38QQR) in recurrent malignant glioma: a report by the Cintredekin Besudotox Intraparenchymal Study Group.* J Clin Oncol, 2007. **25**(7): p. 837-44.
28. Vogelbaum, M.A., et al., *Convection-enhanced delivery of cintredekin besudotox (interleukin-13-PE38QQR) followed by radiation therapy with and without temozolomide in newly diagnosed malignant gliomas: phase 1 study of final safety results.* Neurosurgery, 2007. **61**(5): p. 1031-7; discussion 1037-8.
29. Rand, R.W., et al., *Intratumoral administration of recombinant circularly permuted interleukin-4-Pseudomonas exotoxin in patients with high-grade glioma.* Clin Cancer Res, 2000. **6**(6): p. 2157-65.
30. Lidar, Z., et al., *Convection-enhanced delivery of paclitaxel for the treatment of recurrent malignant glioma: a phase I/II clinical study.* J Neurosurg, 2004. **100**(3): p. 472-9.
31. Hau, P., et al., *Inhibition of TGF-beta2 with AP 12009 in recurrent malignant gliomas: from preclinical to phase I/II studies.* Oligonucleotides, 2007. **17**(2): p. 201-12.
32. Patel, S.J., et al., *Safety and feasibility of convection-enhanced delivery of Cotara for the treatment of malignant glioma: initial experience in 51 patients.* Neurosurgery, 2005. **56**(6): p. 1243-52; discussion 1252-3.
33. Saito, R., et al., *Convection-enhanced delivery of Ls-TPT enables an effective, continuous, low-dose chemotherapy against malignant glioma xenograft model.* Neuro Oncol, 2006. **8**(3): p. 205-14.
34. Saito, R., et al., *Convection-enhanced delivery of tumor necrosis factor-related apoptosis-inducing ligand with systemic administration of temozolomide prolongs survival in an intracranial glioblastoma xenograft model.* Cancer Res, 2004. **64**(19): p. 6858-62.
35. Krauze, M.T., et al., *Convection-enhanced delivery of nanoliposomal CPT-11*

(irinotecan) and PEGylated liposomal doxorubicin (Doxil) in rodent intracranial brain tumor xenografts. Neuro Oncol, 2007. **9**(4): p. 393-403.
36. Yamashita, Y., et al., *Convection-enhanced delivery of a topoisomerase I inhibitor (nanoliposomal topotecan) and a topoisomerase II inhibitor (pegylated liposomal doxorubicin) in intracranial brain tumor xenografts.* Neuro Oncol, 2007. **9**(1): p. 20-8.
37. Dickinson, P.J., et al., *Canine spontaneous glioma: a translational model system for convection-enhanced delivery.* Neuro Oncol, 2010. **12**(9): p. 928-40.
38. Dickinson, P.J., et al., *Canine model of convection-enhanced delivery of liposomes containing CPT-11 monitored with real-time magnetic resonance imaging: laboratory investigation.* J Neurosurg, 2008. **108**(5): p. 989-98.
39. Krauze, M.T., et al., *Safety of real-time convection-enhanced delivery of liposomes to primate brain: a long-term retrospective.* Exp Neurol, 2008. **210**(2): p. 638-44.
40. Valles, F., et al., *Anatomic compression caused by high-volume convection-enhanced delivery to the brain.* Neurosurgery, 2009. **65**(3): p. 579-85; discussion 585-6.
41. Hadjipanayis, C.G., et al., *EGFRvIII antibody-conjugated iron oxide nanoparticles for magnetic resonance imaging-guided convection-enhanced delivery and targeted therapy of glioblastoma.* Cancer Res, 2010. **70**(15): p. 6303-12.
42. MacKay, J.A., D.F. Deen, and F.C. Szoka, Jr., *Distribution in brain of liposomes after convection enhanced delivery; modulation by particle charge, particle diameter, and presence of steric coating.* Brain Res, 2005. **1035**(2): p. 139-53.
43. Wolff, J.E., et al., *Treatment of recurrent diffuse intrinsic pontine glioma: the MD Anderson Cancer Center experience.* J Neurooncol, 2012. **106**(2): p. 391-7.
44. Atadja, P., *Development of the pan-DAC inhibitor panobinostat (LBH589): successes and challenges.* Cancer Lett, 2009. **280**(2): p. 233-41.
45. Schwartzentruber, J., et al., *Driver mutations in histone H3.3 and chromatin remodelling genes in paediatric glioblastoma.* Nature, 2012. **482**(7384): p. 226-31.
46. Wu, G., et al., *Somatic histone H3 alterations in pediatric diffuse intrinsic pontine gliomas and non-brainstem glioblastomas.* Nat Genet, 2012. **44**(3): p. 251-3.
47. Buczkowicz, P., et al., *Histopathological spectrum of paediatric diffuse intrinsic pontine glioma: diagnostic and therapeutic implications.* Acta Neuropathol, 2014. **128**(4): p. 573-81.
48. Buczkowicz, P. and C. Hawkins, *Pathology, Molecular Genetics, and Epigenetics of Diffuse Intrinsic Pontine Glioma.* Front Oncol, 2015. **5**: p. 147.
49. Khuong-Quang, D.A., et al., *K27M mutation in histone H3.3 defines clinically and biologically distinct subgroups of pediatric diffuse intrinsic pontine gliomas.* Acta Neuropathol, 2012. **124**(3): p. 439-47.
50. Korshunov, A., et al., *Integrated analysis of pediatric glioblastoma reveals a subset of biologically favorable tumors with associated molecular prognostic markers.* Acta Neuropathol, 2015.
51. Sturm, D., et al., *Hotspot mutations in H3F3A and IDH1 define distinct epigenetic and biological subgroups of glioblastoma.* Cancer Cell, 2012. **22**(4): p. 425-37.
52. Grasso, C.S., et al., *Functionally defined therapeutic targets in diffuse intrinsic pontine glioma.* Nat Med, 2015. **21**(6): p. 555-9.
53. Rasmussen, T.A., et al., *Activation of latent human immunodeficiency virus by the histone deacetylase inhibitor panobinostat: a pilot study to assess effects on the central nervous system.* Open Forum Infect Dis, 2015. **2**(1): p. ofv037.

54. Gill, T., et al., *In vitro and in vivo testing of a novel recessed-step catheter for reflux-free convection-enhanced drug delivery to the brain.* J Neurosci Methods, 2013. **219**(1): p. 1-9.
55. Matsuo, M., et al., *Effects of intracerebroventricular administration of 2-hydroxypropyl-beta-cyclodextrin in a patient with Niemann-Pick Type C disease.* Mol Genet Metab Rep, 2014. **1**: p. 391-400.
56. Maarup, T.J., et al., *Intrathecal 2-hydroxypropyl-beta-cyclodextrin in a single patient with Niemann-Pick C1.* Mol Genet Metab, 2015. **116**(1-2): p. 75-9.
57. Chittiboina, P., et al., *Magnetic resonance imaging properties of convective delivery in diffuse intrinsic pontine gliomas.* J Neurosurg Pediatr, 2014. **13**(3): p. 276-82.
58. Chittiboina, P., J.D. Heiss, and R.R. Lonser, *Accuracy of direct magnetic resonance imaging-guided placement of drug infusion cannulae.* J Neurosurg, 2015. **122**(5): p. 1173-9.
59. Saito, R., et al., *Regression of recurrent glioblastoma infiltrating the brainstem after convection-enhanced delivery of nimustine hydrochloride.* J Neurosurg Pediatr, 2011. **7**(5): p. 522-6.
60. Barua, N.U., et al., *Robot-guided convection-enhanced delivery of carboplatin for advanced brainstem glioma.* Acta Neurochir (Wien), 2013. **155**(8): p. 1459-65.
61. Anderson, R.C., et al., *Convection-enhanced delivery of topotecan into diffuse intrinsic brainstem tumors in children.* J Neurosurg Pediatr, 2013. **11**(3): p. 289-95.
62. Souweidane MM, Kramer K, Pandit-Taskar N, Zhou Z, Hague S, Zanzonico P, et al. Convection-enhanced delivery for diffuse intrinsic pontine glioma: a single-centre, dose-escalation, phase 1 trial. Lancet Oncol. 2018;19: 1040-50.

APPENDIX A Performance Status Criteria

Karnofsky		**Lansky**	
Score	Description	Score	Description
100	Normal, no complaints, no evidence of disease	100	Fully active, normal.
90	Able to carry on normal activity, minor signs or symptoms of disease.	90	Minor restrictions in physically strenuous activity.
80	Normal activity with effort; some signs or symptoms of disease.	80	Active, but tires more quickly
70	Cares for self, unable to carry on normal activity or do active work.	70	Both greater restriction of and less time spent in play activity.
60	Required occasional assistance, but is able to care for most of his/her needs.	60	Up and around, but minimal active play; keeps busy with quieter activities.
50	Requires considerable assistance and frequent medical care.	50	Gets dressed, but lies around much of the day; no active play, able to participate in all quiet play and activities.
40	Disabled, requires special care and assistance.	40	Mostly in bed; participates in quiet activities.
30	Severely disabled, hospitalization indicated. Death not imminent.	30	In bed; needs assistance even for quiet play.
20	Very sick, hospitalization indicated. Death not imminent.	20	Often sleeping; play entirely limited to very passive activities.
10	Moribund, fatal processes progressing rapidly.	10	No play; does not get out of bed.

APPENDIX B Enzyme Inducing and Recommended Non-Enzyme Inducing Anti-Convulsants; Drugs to avoid

Recommended Non-enzyme inducing anticonvulsants	
Generic Name	*Trade Name*
Gabapentin	Neurontin
Lamotrigine	Lamictal
Levetiracetam	Keppra
Tigabine	Gabitril
Topiramate	Topamax
Valproic Acid	Depakote, Depakene
Zonisamide	Zonegran
Enzyme inducing anticonvulsants	
Generic Name	*Trade Name*
Carbamazepine	Tegretol
Felbamate	Felbatol
Phenobarbital	Phenobarbital
Phenytoin	Dilantin
Primidone	Mysoline
Oxcarbazepine	Trileptal

Drugs to avoid

CYP3A Inhibitors and inducers	*Sensitive substrates for CYP2D6*	*Anti-arrhythmic medicines, including:*	*Drugs that are known to prolong the QT interval, including:*
Ketoconazole Itraconazole Fluconazole Cimetidine Clarithromycin Erythromycin Troleandomycin Grapefruit juice Carbamazepine Rifampin Rifabutin Ritonavir St. John's wort	Atomoxetine Desipramine Dextromethorphan Eliglustat[(e)] Nebivolol Nortriptyline Perphenazine Tolterodine Venlafaxine	Amiodarone Disopyramide Procainamide Quinidine Sotalol	Chloroquine Halofantrine Clarithromycin Methadone Moxifloxacin Bepridil Pimozide

APPENDIX C PNOC Institutions Required Regulatory Documents

Prior to opening a study at any member institution, the following regulatory documents must be submitted to the PNOC Operations Office:

- Participating Site IRB approval(s) for the protocol, appendices, informed consent form and HIPAA authorization
- Participating Site IRB approved consent form
- Participating Site IRB membership list
- Participating Site IRB's Federal Wide Assurance number and OHRP Registration number
- Curriculum vitae and medical license for each investigator and consenting professional
- Documentation of Human Subject Research Certification training for investigators and key staff members at the Participating Site
- Participating site laboratory certifications and normals
- Signed copy of the completed delegation of authority log (found in PNOC Documents > Forms)
- Signed copy of the protocol signature page
- Signed copy of the final contract

Upon receipt of the required documents, the PNOC Operations Office will formally contact the site and grant permission to proceed with enrollment. All documents can uploaded directly to SharePoint by navigating to your site's page and clicking "Add Documents"

Each PNOC site is responsible for ensuring all regulatory documents in SharePoint are up to date. Sites will upload new or revised documents as applicable to reflect any changes, including changes in staff and approved/expired documents.

APPENDIX D Required Data and Time Table for Submission

Form	Submission Timeline
Eligibility Checklist	Complete prior to registration
On Study Forms	Within 14 days of registration
Baseline Assessment Forms	Within 14 days of registration
Treatment Forms	Within 10 days of the last day of the cycle
Adverse Event Report Forms	All AEs are due within 10 business days of the date of assessment.
Serious Adverse Event Reporting	Within 1 business day of first PI awareness
Response Assessment Forms	Within 10 days of the completion of the cycle required for response evaluation
Off Treatment/Off Study Forms	Within 14 days of completing treatment or being taken off study for any reason
Follow up/Survival Forms	Within 14 days of the protocol defined follow up visit date

APPENDIX E PNOC Data and Safety Monitoring

PNOC Data Safety and Monitoring Plan for a Phase 1 Study

It is the responsibility of each PNOC member institution to follow the National Cancer Institute (NCI) approved Data Safety and Monitoring Plan (DSMP) for their site, and to be internally monitored by a Data Safety Monitoring Committee/Board (DSMC/DSMB) or equivalent as approved by the UCSF DSMC. In addition to the guidelines laid out in this document, each PNOC member institution must comply with the policies and standards put forward by their own institutional DSMC/DSMB. For member institutions that do not follow an NCI-approved DSMP, the UCSF DSMC will be considered the "institutional DSMC" mentioned in this document. Such institutions will be electronically monitored.

The institutional DSMC/DSMB activities for this study will include:

- Review of subject data
- Review of suspected adverse reactions considered "serious" (SAEs)
- Monitoring every month/ (depending on patient accrual)
- Minimum of a yearly regulatory audit

Monitoring and reporting guidelines
All institutional Phase 0 or Phase 1 therapeutic studies are designated with a high risk assessment. The data is monitored monthly by the institutional DSMC/DSMB as subjects are enrolled and includes all visits monitored up through the Dose Limiting Toxicity (DLT) period.

The UCSF Helen Diller Family Comprehensive Cancer Center (HDFCCC) DSMC is responsible for monitoring data quality and patient safety for all HDFCCC institutional clinical studies. In the case of all PNOC protocols, the UCSF DSMC will work together with non-UCSF PNOC member institution DSMC/DSMBs in order to ensure DSMP compliance. Each non-UCSF DSMC/DSMB will be responsible for providing regular monitoring reports to PNOC and the UCSF DSMC. These reports will be used by the UCSF DSMC to assess data quality, patient safety, and protocol compliance as well as to make decisions about dose escalations, where applicable.

PNOC and the UCSF DSMC reserve the right to conduct on-site monitoring at any non-UCSF member institution if DSMP requirements are not being met. If the need to perform a monitoring visit at a non-UCSF member institution arises, source documents will be provided by the member institution prior to the visit in order for the UCSF DSMC to monitor protocol compliance, patient safety, and to verify data entry.

The PNOC Operations Office provides administration, data management, and organizational support for the PNOC member institutions in the conduct of any PNOC clinical trial. The PNOC Operations Office will summarize and communicate adverse events, safety data, and other study matters to the PNOC member institutions on a quarterly basis.

The Study Chair is responsible for the overall conduct of any PNOC trial and for monitoring its safety and progress at all participating sites (as outlined in the PNOC Study Chair and Co-Chair Responsibilities SOP). The Study Chair will conduct continuous review of data and subject safety and discuss each subject's treatment with the PNOC Operations Office. The discussions are documented in the PNOC Operations Office meeting minutes.

Multicenter communication

The PNOC Operations Office will coordinate, at minimum, quarterly conference calls with the PNOC member institutions to discuss risk assessment. The following items will be discussed, as appropriate:

- Enrollment information
- Cohort updates (e.g. DLTs and dose escalations)
- Adverse Events (e.g. new AEs, unresolved AEs, and new safety information)
- Protocol violations
- Other study conduct issues

Dose level considerations
Dose level assignments for any subject scheduled to begin treatment **must be confirmed** by the PNOC Operations Office via e-mail.

If an unexpected Dose Limiting Toxicity (DLT) occurs in a subject treated at any participating PNOC member institution, all member institutions must be notified of the DLT by the PNOC Operations Office within **1 business day**. Member institutions will otherwise be provided with final DLT reports as they become available.

Adverse event review and monitoring
PNOC uses the web-based OnCore® Clinical Trials Management System for all patient registrations and data entry. The OnCore® System will also track patient level protocol compliance and safety information.

All Adverse Events (AEs) will be entered into OnCore®.

All Adverse Events entered into OnCore® will be reviewed on a weekly basis by the PNOC Operations Office. The PNOC Operations Office will discuss the toxicity, grade, and relationship to study intervention for all AEs in question.

All Adverse Events must be entered into OnCore® within **10 business days** of becoming aware of the event. Member institutions will submit this information to PNOC via the Adverse Event Form within OnCore®.

In addition, all adverse reactions considered “serious” (also called Serious Adverse Events, or SAEs), regardless of relationship, must be entered in OnCore® and reported to the PNOC Operations Office within **1 business day**. SAEs will be reviewed and monitored by the UCSF DSMC on an ongoing basis, and will be discussed at the UCSF DSMC meeting, which take place every six (6) weeks.

If a death occurs during the treatment phase of the study, or within 30 days after the last administration of the study drug(s), and is determined to be related either to the investigational drug or to any research related procedure, the Study Chair and the PNOC Operations Office must be notified by the member institution within **1 business day.** The Study Chair or the PNOC Operations Office must then notify the UCSF DSMC Chair, or qualified alternate, within 1 business day of this notification. The contact may be by phone or e-mail.

Increase in adverse event rates
If an increase in the frequency of Grade 3, 4, or 5 Adverse Events (above the rate reported in the Investigator Brochure or package insert), the Study Chair or the PNOC Operations Office is responsible for notifying the UCSF DSMC at the time the increased rate is identified.

If at any time the Study Chair or the PNOC Operations Office halts enrollment or ends the study due to safety issues, the UCSF DSMC Chair and Manager must be notified within **1 business day** via e-mail. The UCSF DSMC must receive a formal letter within **10 business days,** and the UCSF IRB must be notified.

UCSF data and safety monitoring committee contacts:

UCSF DSMC Chair
[redacted]
UCSF-Box #1705
San Francisco, CA 94115

UCSF DSMC Manager
[redacted]
UCSF-Box #0128
San Francisco, CA 94143

UCSF DSMC Monitors
UCSF-Box #0128
San Francisco, CA 94143

APPENDIX F Information Sheet on Possible Drug Interactions

Information on Possible Interactions with Other Agents for Patients and Their Caregivers and Non-Study Healthcare Team

The patient _____ is enrolled on a clinical trial using the experimental agent **[agent name]**. *This clinical trial is sponsored by the Pacific Pediatric Neuro-Oncology Consortium and [Sponsor-Pharmaceutical Company]. This form is addressed to the patient, but includes important information for others who care for this patient.*

[Agent name] interacts with many drugs that are processed by your liver. Because of this, it is very important to tell your study doctors about all of your medicine before you start this study. It is also very important to tell them if you stop taking any regular medicine, or if you start taking a new medicine while you take part in this study. When you talk about your medicine with your study doctor, include medicine you buy without a prescription at the drug store (over-the-counter remedy), or herbal supplements such as St. John's wort.

Many health care prescribers can write prescriptions. You must also tell your other prescribers (doctors, physicians' assistants or nurse practitioners) that you are taking part in a clinical trial. **Bring this paper with you and keep the attached information card in your wallet**. These are the things that you and they need to know:

[Use or delete sections below as appropriate.]

[Agent name] interacts with (a) certain specific enzyme(s) in your liver.

- The enzyme(s) in question is/are ***[name(s) of CYP isoenzyme(s)]***, and *[insert brief, easy explanation of the nature of the interaction,* i.e., *for inducers: "[agent name] is broken down by this enzyme in order to be cleared from your system."]*
- *[Agent name]* must be used very carefully with other medicines that need these liver enzymes to be effective or to be cleared from your system.
- Other medicines may also affect the activity of the enzyme.
    - *[The following text is for agents that are metabolized/cleared by the enzyme.]* Substances that increase the enzyme's activity ("inducers") could reduce the effectiveness of the drug, while substances that decrease the enzyme's activity ("inhibitors") could result in high levels of the active drug, increasing the chance of harmful side effects.
    - *[The following text is for when the agent requires the enzyme in order to be converted from prodrug to active drug.]* Substances that increase the enzyme's activity ("inducers") could result in high levels of the active drug, increasing the chance of harmful side effects, while substances that decrease the enzyme's activity ("inhibitors") could reduce the effectiveness of the drug.
    - *[The following text is for when the study agent modulates the enzyme activity.] [Agent name]* is considered a(n) "[inducer/inhibitor]"of the enzyme, meaning that it can affect the levels of other drugs that are processed by that enzyme. This can lead to harmful side effects and/or reduce the effectiveness of those medications.
- You and healthcare providers who prescribe drugs for you must be careful about adding or removing any drug in this category.

- Before you start the study, your study doctor will work with your regular prescriber to switch any medicines that are considered "strong inducers/inhibitors or substrates of ***[name(s) of CYP isoenzyme(s)]***."
- Your prescribers should look at this web site http://medicine.iupui.edu/clinpharm/ddis/table.aspx or consult a medical reference to see if any medicine they want to prescribe is on a list of drugs to avoid.
- Please be very careful! Over-the-counter drugs have a brand name on the label—it's usually big and catches your eye. They also have a generic name—it's usually small and located above or below the brand name, and printed in the ingredient list. Find the generic name and determine, with the pharmacist's help, whether there could be an adverse interaction.
- *[The following are **examples** of text for common over-the-counter medications or supplements that may interact with the study agent.]* Be careful:
    - If you take acetaminophen regularly: You should not take more than 4 grams a day if you are an adult or 2.4 grams a day if you are older than 65 years of age. Read labels carefully! Acetaminophen is an ingredient in many medicines for pain, flu, and cold.
    - If you drink grapefruit juice or eat grapefruit: Avoid these until the study is over.
    - If you take herbal medicine regularly: You should not take St. John's wort while you are taking *[agent name]*.
    - *[Add other specific medications here, if necessary.]*

Other medicines can be a problem with your study drugs.

- You should check with your doctor or pharmacist whenever you need to use an over-the-counter medicine or herbal supplement.

- Your regular prescriber should check a medical reference or call your study doctor before prescribing any new medicine for you. Your study doctor's name is _____ and he or she can be contacted at

INFORMATION ON POSSIBLE DRUG INTERACTIONS

You are enrolled on a clinical trial using the experimental agent **_____.** This clinical trial is sponsored by the NCI. **_____** interacts with drugs that are processed by your liver. Because of this, it is very important to:

- Tell your doctors if you stop taking regular medicine or if you start taking a new medicine.
- Tell all of your prescribers (doctor, physicians' assistant, nurse practitioner, pharmacist) that you are taking part in a clinical trial.
- Check with your doctor or pharmacist whenever you need to use an over-the-counter medicine or herbal supplement.

_____ interacts with a specific liver enzyme called **CYP_____**, and must be used very carefully with other medicines that interact with this enzyme.

- Before you start the study, your study doctor will work with your regular prescriber to switch any medicines that are considered "strong inducers/inhibitors or substrates of **CYP_____**."
- Before prescribing new medicines, your regular prescribers should go to http://medicine.iupui.edu/clinpharm/ddis/table.aspx for a list of drugs to avoid, or contact your study doctor.
- Your study doctor's name is **_____**

  and can be contacted at **_____.**

APPENDIX G Age Appropriate Blood Pressure and Heart Rate Measures

Blood Pressure for Children by Age and Height

Blood Pressure Levels for **Boys**

Age, y	BP Percentile	SBP, mm Hg							DBP, mm Hg						
		Percentile of Height							Percentile of Height						
		5th	10th	25th	50th	75th	90th	95th	5th	10th	25th	50th	75th	90th	95th
1	95th	98	99	101	103	104	106	106	54	54	55	56	57	58	58
2	95th	101	102	104	106	108	109	110	59	59	60	61	62	63	63
3	95th	104	105	107	109	110	112	113	63	63	64	65	66	67	67
4	95th	106	107	109	111	112	114	115	66	67	68	69	70	71	71
5	95th	108	109	110	112	114	115	116	69	70	71	72	73	74	74
6	95th	109	110	112	114	115	117	117	72	72	73	74	75	76	76
7	95th	110	111	113	115	117	118	119	74	74	75	76	77	78	78
8	95th	111	112	114	116	118	119	120	75	76	77	78	79	79	80
9	95th	113	114	116	118	119	121	121	76	77	78	79	80	81	81
10	95th	115	116	117	119	121	122	123	77	78	79	80	81	81	82
11	95th	117	118	119	121	123	124	125	78	78	79	80	81	82	82
12	95th	119	120	122	123	125	127	127	78	79	80	81	82	82	83
13	95th	121	122	124	126	128	129	130	79	79	80	81	82	83	83
14	95th	124	125	127	128	130	132	132	80	80	81	82	83	84	84
15	95th	126	127	129	131	133	134	135	81	81	82	83	84	85	85
16	95th	129	130	132	134	135	137	137	82	83	83	84	85	86	87
17	95th	131	132	134	136	138	139	140	84	85	86	87	87	88	89

Blood Pressure Levels for **Girls**

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

Instructions for using this BP Chart:

1. Measure the patient's blood pressure using an appropriate size cuff.
2. Select appropriate chart for a female or male patient.
3. Using the "age" row and "height" column to determine if the BP is within the ULN.

These tables were taken from "The Fourth Report on the Diagnosis, Evaluation, and Treatment of High Blood Pressure in Children and Adolescents" PEDIATRICS Vol. 114 No. 2 August 2004, pp. 555-576.

Heart Rate Normal Range

Age	Heart Rate (bpm)
0–7 days	95–160 (125)
1–3 wk	105–180 (145)
1–6 mo	110–180 (145)
6–12 mo	110–170 (135)
1–3 yr	90–150 (120)
4–5 yr	65–135 (110)
6–8 yr	60–130 (100)
9–11 yr	60–110 (85)
12–16 yr	60–110 (85)
>16 yr	60–100 (80)

APPENDIX H Imaging Guidelines for PNOC Studies

Pre-Study Imaging Qualification

The most critical aspect of the advanced imaging being performed in this study is to match quantitative exam protocols prior to the initial treatment and at follow-up studies, so that direct comparisons of intra-patient parameters can be made. Each PNOC site must be satisfied that the anatomic imaging sequences being used at these times satisfy clinical criteria for evaluating their patients. Hence, while there should be an attempt to make the protocols as similar as possible between institutions, it may not be feasible for them to be identical, and so any comparisons that are being made will focus on changes within the patient rather than differences among individuals. Please note that the radiologist at each PNOC site should interpret the anatomic images for clinical purposes and then send them to UCSF for quantitative analysis.

All images generated for each patient should be anonymized and sent to UCSF, either electronically or by CD so that they can be evaluated and confirmed that the protocol satisfies the requirements of the study. Sites should batch ship all images pertaining to each patient at the time the patient comes off treatment.

Guidelines for Imaging Protocols

Serial exams should be performed on the same 3T MR system using the commercial 8-channel or other multi-channel head coil. The sequences may either be performed in a pure axial orientation or aligned with the AC-PC line, as is the default at many institutions. Our guidelines follow the Consensus recommendations for a standardized Brain Tumor Imaging Protocol in clinical trials (Ellingson BM. *Neuro-Oncology*. 2015).

Recommended outline of MR imaging protocol:

1. 3-plane localizer
2. T1-weighted pre-gadolinium volumetic images: this high-resolution (3-dimensional 1 mm isotropic) acquisition is used as a reference for comparing with the post-gadolinium images and to identify signs of hemorrhage. We recommend 3-dimensional isotropic T1-weighted images using a gradient-recalled echo (GRE) acquisition with inversion preparation (IR)
3. T2-weighted images: used in conjunction with the T2-weighted fluid-attenuated inversion recovery (FLAIR) images to define the spatial extent of the T2 lesion.
4. FLAIR images: required for defining treatment response using the RANO criteria or iRANO criteria as indicated per each study protocol.
5. Diffusion weighted images: the entire brain should be covered with at least 6 different gradient directions at b=1000 and with one acquisition having b=0. The slice thickness and spatial resolution should be chosen to allow calculation of maps of apparent diffusion coefficient and fractional anisotropy.
6. Echo planar gradient echo dynamic susceptibility contrast (DSC) images: A series of images should be acquired during the injection of a bolus of 0.1mmol/kg of gadolinium contrast agent that is delivered at a rate of 3-5m/s using a power injector and with a 15-20ml flush of normal saline delivered at the same rate. The dose and timing of gadolinium should be kept consistent to facilitate clinical interpretation. Slice thickness (3-5mm) and location should be chosen to cover as much of the T2 lesion as possible.

The injector delay should be set at 15-30s to allow a good definition of baseline intensities from the pre-bolus images. Additionally, the dose and agent should be explicitly documented on the MR system during acquisition or labeled in the DICOM header.

7. Post-gadolinium T1-weighted volumetric images: this high-resolution (3-dimensional 1 mm isotropic) acquisition is used to define the spatial extent of the enhancing volume and for registration between examinations. We recommend 3-dimensional isotropic T1-weighted images using a gradient-recalled echo (GRE) acquisition with inversion preparation (IR).

8. Post-gadolinium T1-weighted images: these should match the pre-gadolinium images are used to define the extent of the enhancing lesion. For consistency in contrast enhancement we recommend that these images be acquired *after* volumetric post-gadolinium T1-weighted images.

Any of the above sequences or a combination thereof may be used for quantitative analysis of disease response and/or treatment effect. Decisions regarding which sequences will be utilized will be determined as based on the specific study intervention and anticipated imaging findings that accompany the intervention (e.g. immunotherapy vs. targeted small molecules), as well as individual characteristics of tumor subtypes. In addition to assessing disease response and treatment effect, sequences may be used for pre-surgery exams, clinical evaluation of the patient, and volumetric analysis of regions of interest.

De-Identification and Labeling

It is the responsibility of the PNOC sites to de-identify images according to HIPAA, Institutional Review Board (IRB) guidelines, GCPs and local regulatory requirements, with the following considerations:

- Do not remove the date of the exam or the technical information (eg, slice location, kVP, echo time, etc).
- Do not modify time or date information before or during the de-identification process.

Labeling of Digital Images

Use the patient ID, the date (ddmmyy) and scan number (01 or 02 for the two advanced imaging exams) to label the data as follows: PatientID_date_xx

Checklist for Media Submission

- Completed Exam Data Sheet.
- De-identified DICOM images.

Sending Digital Images via FedEx:

- PNOC sites should batch ship all images pertaining to each patient at the time the patient comes off treatment.
- All MRIs should be de-identified prior to shipment.
- All shipping materials should be provided by the site.
- All shipments require signature for delivery.

- Include checklist items and address packages sent to the PNOC Operations Office as outlined in the Imaging SOP on the PNOC SharePoint website.

Example of Data Analysis Performed by PNOC Central Review

The anatomic images will be used to manually define the contrast enhancing lesion (CEL) and the T2 lesion (T2L), as well as T2/FLAIR changes. The T1 weighted pre-contrast image will be used to define a brain mask so that intensity values can be normalized. The diffusion images are processed to generate maps of apparent diffusion coefficient (ADC) and fractional anisotropy (FA). The perfusion data are processed to calculate maps of relative cerebral blood volume (rCBV), peak height (PH) and percentage recovery (RECOV).