Phase Ib study of Oncolytic Polio/Rhinovirus Recombinant Against Recurrent Malignant Glioma in Children

Sponsor:	Istari Oncology, Inc.
Funding Source:	Solving Kids Cancer in collaboration with The Andrew McDonough B+ Foundation
Protocol Source:	Duke Cancer Institute
Duke IRB#:	Pro00071228
WIRB#:	20171431
IND#	14,735

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Revision History

Original version:	02/24/2016	
Amended version 1:	10/24/2016	Revisions post RAC review
Amended version 2:	12/23/2016	Revisions post CPC review
Amended version 3:	03/09/2017	Change in Dose Level
Amended version 4:	05/16/2017	Change in IND Sponsor
Amended version 4.1:	08/04/2017	Revisions post WIRB review
Amended version 5:	10/10/2017	Added David Ashley contact information. Added WIRB protocol number to title page. Added three additional core biopsies for full genome and full exome sequencing (Sections 9.1.1, 9.1.4, 12.3). Edited dose error in Section 9.2. Removed stool sampling at 8 weeks based on the IBC decision that it was no longer necessary (Section 9.1.6, 12, and 12.4**Error! Reference source not found.**). Removed reference to Theratаxis as they will no longer be used to review MRIs (Section 9.1.3). Clarified that the study will include 18 year old patients (Section 7.3 and 11.1). Added patient and guardian in addition to parents as able to sign consent in the Inclusion Criteria and changed SGOT/SGPT to AST/ALT (Section 11.1). SGOT=AST and SGPT=ALT, but we commonly use the term AST/ALT in our clinic, so the wording has been updated. Monitoring rules updated to monitoring occurring after each of the first 3 patients, followed by annual monitoring of 1-3 patients (Section 14.1).
Amended version 6:	12/11/2017	Minor corrections or revisions (Sections 5.2, 9.1.2, 9.1.6). Updates to the study drug information (Sections 10.3, 10.4). Remove “unsupported” from 2 laboratory inclusion criteria (Section 11.1). Changes or corrections made to schedule of procedures in Table 2 and throughout Section 12. Changes to blood volumes in Table 3 and throughout Section 12.
Amended version 7:	03/06/2018	Withdrawn/not implemented
Amended version 8:	07/30/2018	Revisions to inclusion and exclusion criteria in Sections 11.1 and 11.2 to allow for a greater number of potentially eligible patients. Rationale provided in Section 7.3. Additional information regarding use of bevacizumab in the pediatric population has been added to Section 9.1.6.
Amended version 8.1:	09/18/2018	Revisions requested by FDA to v.8.0 inclusion criteria #1 and #2 in Section 11.1.
Amended version 9:	11/21/2018	Remove rationale for including patients with lesions in the posterior fossa in Section 7.3. Revisions to eligibility criteria to add ‘supratentorial’ to inclusion criterion #1 in Section 11.1 and add ‘cerebellum’ to exclusion criterion #10 in Section 11.2. Additional instructions for safety reporting (SAE reporting within 24h of PI/site learning of event) to the Sponsor provided in Section 13.2.1.
Amended version 10:	02/20/2019	Change Co-PI to Daniel Landi, MD; add David Ashley, MBBS, FRACP, PhD as sub-investigator
Amended version 11:	10/31/2019	Updated sub-investigators and changed Lead Study Coordinator on p.1; Rationale for

		expanding the eligible age to a younger patient population added to Section 7.3. Re-wording in Section 7.2.4. Clinical experience background information updated in Section 7.2.6. Magnevist® co-infusion removed from study, along with corresponding immediate post-infusion MRI affecting Sections 5.3, 9.1.2, 9.1.3, 11.2, and 12.3 and Table 2. Sentence removed from Section 9.1.3. Sentence describing risks of sedation added in Section 9.1.6; Age range changed in Section 9.2 and in inclusion criteria in Section 11.1. Inclusion criterion regarding ability to undergo brain MRI without anesthesia removed in Section 11.1. Clarified assent in inclusion criteria in Section 11.1; Exclusion criterion regarding previous PV infection re-worded in Section 11.2; Exclusion criterion regarding gadolinium allergy changed in Section 11.2; Remove KPS on Day 1 in Section 12, Table 2 and Section 12.4; Add a 4 mL blood draw for immune analysis on Day 1 in Section 12, Table 2 and Section 12.4; Baseline MRI within 3 days changed to within 5 days in Table 2 and Section 12.1; Text in Section 13.1.1 changed to N/A.
Amendment Version 12	08/19/2020	Cover page: updated CMO for Istari. Updated language to remove 14-day hold between treated patients based on interim safety review in Sections 5.5, 7.3, and 15.4.1. Clarified Sponsor monitoring in Section 14.1.

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aPTT	Activated Partial Thromboplastin Time
AE	Adverse Event
ALK	Alkaline Phosphatase
ALT	Alanine Transaminase
AST	Aspartate aminotransferase
ATRT	Atypical Teratoid/Rhabdoid Tumor
CBC	Complete Blood Count
CED	Convection-enhanced Delivery
CMP	Comprehensive Metabolic Panel
CNS	Central Nervous System
CPC	Cancer Protocol Committee
CR	Complete Response
CRM	Continual Reassessment Method
CT	Computed Tomography
CTCAE	Common Terminology Criteria for Adverse Events
DLT	Dose Limiting Toxicity
DMEM	Dulbecco's Minimal Essential Medium
DSMB	Data and Safety Monitoring Board
DVT	Deep Vein Thrombosis
EGFRvIII	Epidermal Growth Factor Receptor (variant III)
eIF	Eukaryotic Initiation Factor
FDA	Food and Drug Administration
FLAIR	Fluid-Attenuated Inversion Recovery
GBM	Glioblastoma
GCS	Glascow Coma Scale
Gd-DTPA	Gadolinium Diethylene Triamine Pentaacetic Acid
H&P	History & Physical Exam
HRPP	Human Research Protections Program
HRV2	Human Rhinovirus Type 2
HAS	Human Serum Albumin
IgG	Immunoglobulin G
ICS	Investigational Chemotherapy Service
IND	Investigational New Drug
IRB	Institutional Review Board
IRES	Internal Ribosomal Entry Site
KPS	Karnofsky Performance Status
ICH	Intracerebral Hemorrhage
IV (or iv)	Intravenously
LSQ	Lymphocyte Subset Quantitation
MG	Malignant Glioma
MMSE	Mini-Mental State Exam
NCI	National Cancer Institute
Necl-5	Nectin-like Molecule 5
NED	No Evidence of Disease
NHP	Non-human Primate
NINDS	National Institute of Neurological Disease & Stroke
NSICU	Neuro-Surgical Intensive Care Unit
OARC	Office of Audit, Risk, and Compliance
ORR	Overall Response Rate
OS	Overall Survival
PD	Progressive Disease
PET	Positron Emission Tomography
PFS	Progression Free Survival
PKC	Protein Kinase C

p.o.	per os/by mouth/orally
PR	Partial Response
PRTBTC	Preston Robert Tisch Brain Tumor Center
PT	Prothrombin Time
PV	Poliovirus
PV1S	Poliovirus Serotype 1 (Sabin)
PVSRIPO	Polio/Rhinovirus Recombinant
RAC	Recombinant DNA Advisory Committee
SAE	Serious Adverse Event
SD	Stable Disease
SIADH	Syndrome of Inappropriate Antidiuretic Hormone
TCID	Tissue Culture Infectious Dose
UTR	Untranslated Region
WHO	World Health Organization

5 PROTOCOL SYNOPSIS AND RESEARCH SUMMARY

5.1 Purpose

The purpose of the study is to confirm the safety of the selected dose and potential toxicity of oncolytic poliovirus (PV) immunotherapy with PVSRIPO for pediatric patients with recurrent WHO grade III or IV malignant glioma, but evidence for efficacy will also be sought.

Primary Objective:
1. Confirm the safety of the selected dose of PVSRIPO when delivered intracerebrally by convection-enhanced delivery (CED) in children with recurrent WHO Grade III malignant glioma (anaplastic astrocytoma, anaplastic oligoastrocytoma, anaplastic oligodendroglioma, anaplastic pleomorphic xanthoastrocytoma, ependymoma) or WHO Grade IV malignant glioma (glioblastoma, gliosarcoma)

Secondary Objective:
1. Estimate Overall Survival (OS) in children with recurrent WHO grade III and IV malignant glioma treated with a single dose of PVSRIPO

Exploratory Objectives:
1. Describe changes visualized on imaging due to intratumoral inoculation of PVSRIPO
2. Describe changes in tumor volume visualized on imaging after intratumoral inoculation of PVSRIPO
3. Assess immunologic responses in peripheral blood and in serum
4. Identify genetic predictors of response or failure of response to treatment with PVSRIPO

Hypothesis:
Intracerebral injection of PVSRIPO will be feasible and safe in children with WHO grade III or IV malignant glioma.

5.2 Background and Significance

Pediatric brain tumors are the most common solid tumor in children with approximately 4,000 cases every year in the United States. While significant improvements have been made in the treatment of these children, the outcome for those with recurrent brain tumors remains grim. This is particularly so for those with recurrent malignant glioma. Treatment failure is frequently due to the presence of a blood-brain barrier resulting in poor penetration of cytotoxic drugs into areas where this barrier is intact and poor tumor control. Various approaches have been used successfully to circumvent the blood-brain barrier, including CED, a process by which large molecules (>400 daltons) are directly infused under pressure into a tumor through a catheter. CED results in adequate distribution of such molecules into the tumor over large areas via inherent interstitial fluid pathways.

Oncolytic virus immunotherapy for brain tumors is a unique approach with several advantages over more conventional drugs. Certain oncolytic viruses are capable of selective tumor cell killing with a range of inflammatory and immune-stimulatory effects on the tumor itself, tumor stromal component and the host immune system at large. The objective of oncolytic immunotherapy is to recruit effector adaptive immune responses against tumor-associated antigens that can produce lasting immunologic control of cancers.

PVSRIPO is a genetically recombinant, non-pathogenic PV:rhinovirus chimera with a tumor-specific conditional replication phenotype. It consists of the genome of the live attenuated PV serotype 1 (Sabin) vaccine (PV1S) with its cognate IRES element replaced with that of HRV2. PVSRIPO tumor tropism is mediated by the PV receptor, CD155. CD155, an onco-fetal cell adhesion molecule ectopically upregulated in ectodermal/ neuroectodermal cancers, is broadly expressed on cancerous cells, cancer ‘stem-cell-like cells’ and tumor-associated proliferating vasculature. Infection with PVSRIPO results in swift destruction of tumor cells. PV’s inherent neuropathogenicity was removed by IRES exchange; this ablated the virus’ ability to propagate in cells of neuronal lineage and to

cause poliomyelitis. However, PVSRIPO replicates efficiently in cancerous cells and exhibits potent anti-neoplastic effects in animal tumor models. Tumor cell-specificity mediated by the foreign IRES in PVSRIPO relies (i) on a 'non-productive' ribonucleoprotein complex forming at the foreign HRV2 IRES in neurons; (ii) constitutive signal transduction via PKC-Ras-ERK1/2 to translation machinery, which stimulates viral, cap-independent translation via the HRV2 IRES in cancerous cells.

5.3 Design and Procedure

This protocol is designed primarily to confirm the safety of the selected dose of a novel oncolytic agent with MG-specific viral translation and cytotoxicity. PVSRIPO will be delivered intratumorally by CED using an intracerebral catheter placed within the enhancing portion of the tumor. Based on a Phase I study of the agent in adult patients with recurrent glioblastoma, the amount to be delivered will be 5×10^7 tissue culture infectious dose (TCID50). A total of 3 mLs of the agent in physiologic saline stabilized with 0.2% human serum albumin will be delivered over 6 hours 30 minutes, corresponding to a flow-rate of 0.5 mL/hr. The agent is stable at room temperature during the instillation period and there is no adsorptive loss of PVSRIPO in the intended delivery apparatus.

5.4 Selection of Subjects

All inclusion/exclusion criteria may be found in Section 11.

5.5 Data Analysis and Statistical Considerations

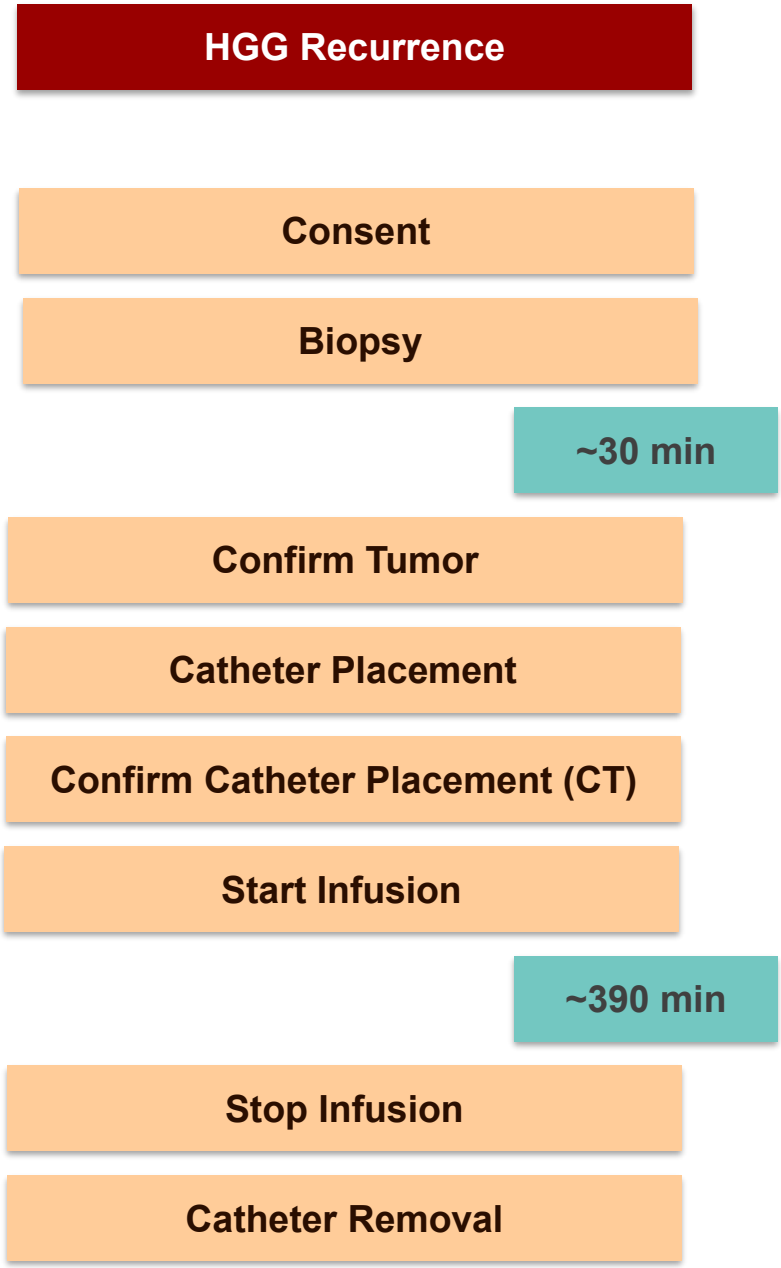
A maximum of 12 patients will be treated with PVSRIPO, and then carefully monitored for safety for at least a year after treatment. Of particular interest will be the incidence of adverse events that occur during the first 14 days after PVSRIPO treatment and the inflammatory events that occur during the first year after PVSRIPO treatment. Our experience with PVSRIPO treatment of adult patients with recurrent WHO grade IV malignant glioma suggests that a patient remains at risk, during the first 12 months post-PVSRIPO treatment, of demonstrating neurologic or radiographic signs suggestive of an inflammatory reaction secondary to the immune response triggered by PVSRIPO that requires a dose of dexamethasone above 4 mg a day. As described in Section 9.1.6, such patients will be treated with bevacizumab 7.5 mg/kg IV every 3 weeks if possible, or alternatively corticosteroids or surgery.

The accrual of pediatric patients to this study was staggered for the first 5 patients enrolled so that no more than 1 patient was treated every 14 days. A slow accrual was planned to minimize the number of pediatric patients exposed to PVSRIPO before adequate information about the sequelae of the inflammatory process within a pediatric patient is known. Based on a review of the available safety data and acceptable tolerability in the first 5 patients treated with PVSRIPO (no dose-limiting toxicities or suspected unexpected serious adverse reactions observed), the protocol was amended to allow for accrual of subsequent patients without the 14-day waiting period in Protocol Amendment 12.

Two sets of safety monitoring guidelines are proposed in Section 15.4.1, with one focused on acute toxicity (i.e. occurring during the first 14 days after PVSRIPO administration), and one focused on long-term issues.

The Kaplan-Meier estimator will describe the survival of patients treated with PVSRIPO. Survival (OS) will be calculated as the time between PVSRIPO infusion and death, or last follow-up if the patient remains alive. Analyses will be stratified by grade.

6 STUDY SCHEMA



7 BACKGROUND AND SIGNIFICANCE

7.1 Study Disease

The brain is the most frequent site for the occurrence of primary solid tumors in children; mortality from this disease has overtaken leukemias and is now the most frequent cause of cancer death in this population [1]. Malignant glioma (including WHO grades III and IV) is the third most common primary CNS malignancy in children, but far less frequent in this age group as compared to adults. The incidence is 0.59 per 100,000 children in the United States with ~200-300 new cases per year [2]. The standard of care treatment for those with newly diagnosed disease includes adequate surgical resection followed by focal radiotherapy and chemotherapy [1]. The use of oral temozolomide concurrently with radiotherapy followed by maintenance chemotherapy using the same agent did not show improved survival compared to historical controls [3]. In a recent Children's Oncology Group (COG) phase II randomized study, the addition of novel agents including bevacizumab or a histone deacetylase (HDAC) inhibitor (Vorinostat; Zolinza™, Merck Corporation, USA) did not show any improvement in survival compared to temozolomide alone resulting in early termination of the study[1]. Once tumor recurs, salvage therapies are often unsuccessful. Treatment failure is frequently due to the presence of a blood-tumor barrier and poor penetration of cytotoxic drugs into tumor [4]. The blood-tumor barrier can be bypassed by CED [4-6]. This therapeutic approach has been widely tested in adults and has also been found to be feasible and safe in children in both cerebral hemispheres and brain stem locations [7].

7.2 Study Agent

7.2.1 PVSRIPO

PVSRIPO is based on the prototype neurovirulent poliovirus (PV) serotype 1 (Mahoney) (**Figure 1A**), which is derived from a stool isolate obtained from a non-symptomatic carrier in Cleveland in the 1940s. Throughout the 1940s, PV type 1 (Mahoney) was subjected to serial passage, either in non-human primates or in tissue culture cells derived from various non-human primate tissues, which yielded the type 1 live-attenuated (Sabin) vaccine strain (PV1S; **Figure 1B**). PV1S is the preferred agent for routine, infant vaccination in the world. The PV1S vaccine was modified by exchange of its internal ribosomal entry site (IRES) with its counterpart from human rhinovirus type 2 (HRV2), generating PVSRIPO (**Figure 1C**). PVSRIPO is characterized by loss of the inherent neurovirulence of poliovirus [8-11].

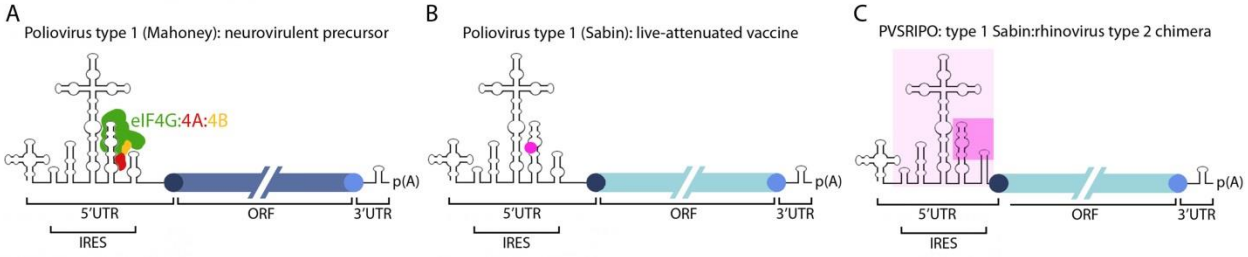


Figure 1. The genetic structure of PVSRIPO (C) and its precursors type 1 (Mahoney) (A), and PV1S (B).

7.2.2 PVSRIPO Oncolysis

PVSRIPO's anti-neoplastic potential is due to a series of (i) direct lytic effects on tumor cells; (ii) presentation of tumor-associated antigens in a highly adjuvanted context; (iii) pro-inflammatory and danger signals stemming from tumor destruction and activation of an antiviral type 1 interferon response; (iv) infection and pro-inflammatory activation of dendritic cells and tumor associated macrophages; (v) durable anti-tumor immunity evoked by effector cytotoxic T lymphocyte responses [12] (**Figure 2**). The principal elements determining PVSRIPO tumor tropism, tumor-specific cell killing, neuronal incompetence/safety, and immunogenicity are well established empirically [13-15].

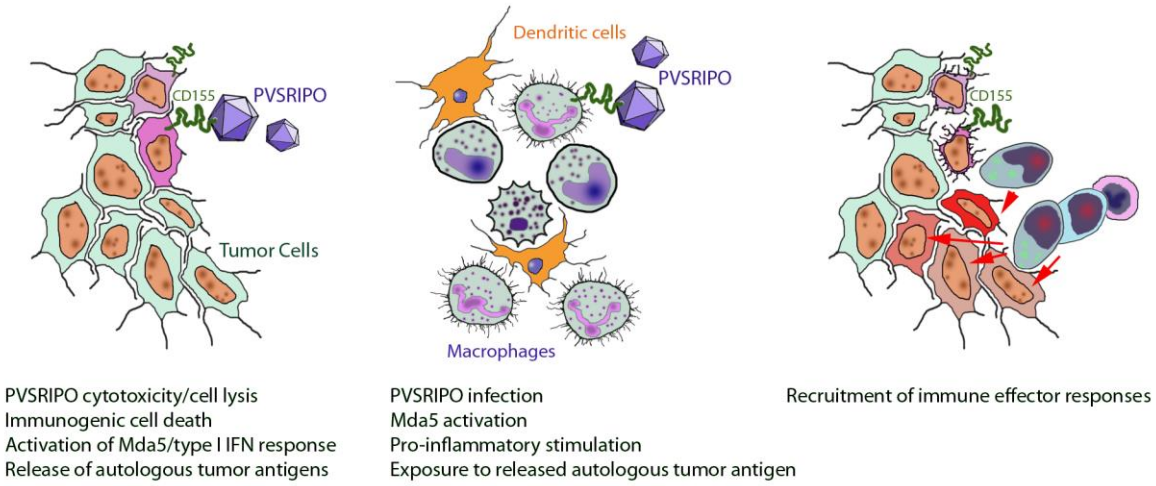


Figure 2. Pathways involved in PVSRIPO oncolytic immunotherapy. PVSRIPO oncolytic immunotherapy is based on combined cytotoxic and immunogenic effects on tumor cells (left), pro-inflammatory effects on antigen-presenting cells (middle) and the generation of adaptive anti-tumor immunity (right).

7.2.3 PVSRIPO Tumor Tropism

Every aspect of PVSRIPO oncolytic immunotherapy is dominated by its relationship with its host cell receptor, CD155 [16]. CD155 is broadly expressed in neuroectodermal malignancies, e.g. MG. Its expression has been empirically confirmed in laboratory MG cell lines, primary patient explant MG cells, primary patient explant MG xenografts maintained in athymic mice, and patient MG tissues obtained during surgery [17]. CD155 is expressed on tumor cells proper, on proliferating tumor vascular cells, and on macrophages and dendritic cells [18,19].

7.2.4 PVSRIPO Tumor-Specific Cell Killing

Infection of tumor cells with PVSRIPO results in their swift destruction, due to very early translation of highly cytotoxic viral proteins immediately after entry of the viral RNA into infected host cells [20]. PVSRIPO achieves this through an unorthodox mechanism of translation initiation [21]. At eukaryotic mRNAs, protein synthesis occurs upon recruitment of a multi-partite protein complex at the canonical 5' 'cap' structure. Poliovirus RNAs are un-capped and, hence, rely on cap-independent translation for viral protein synthesis. This involves direct recruitment of ribosomal subunits via binding of the eukaryotic initiation factor (eIF) 4G to viral RNA (**Figure 1**) [22]. eIF4G-binding occurs via the IRES, a *cis*-acting genetic element located in the 5' untranslated region of the viral RNA. In tumor cells, this process is highly efficient, due to un-hinged signal transduction networks that greatly favor ribosome recruitment to viral RNA [23].

There has been no evidence of PVSRIPO causing infectious polio; PVSRIPO does not infect normal brain cells and is incapable of driving viral translation in normal brain cells. [8] This is due to profound neuronal incompetence of the foreign HRV2 IRES in its genome. The foreign HRV2 IRES cannot recruit eIF4G to the viral genome, precluding viral protein synthesis in neuronal cells/the normal brain [24]. By blocking this crucial, early step in the infectious cycle specifically in neurons, PVSRIPO neurovirulence is eliminated; therefore, PVSRIPO has been deemed acceptable for intracerebral inoculation.

7.2.5 Pre-clinical Experience

In animal tumor models, oncolytic polioviruses elicit efficient anti-neoplastic effects resulting in tumor regression and, eventually, destruction [20]. There is histologic evidence for direct, virus-mediated tumor cell killing and indirect, host-mediated inflammatory responses directed against tumor. PVSRIPO was subjected to extensive dose-range finding, toxicology, biodistribution, shedding and neutralizing antibody tests with intrathalamic inoculation of up to 5 x 10^9 TCID50

of PVSRIPO in *M. Fascicularis* [25]. These revealed: (i) absence of morbidity and mortality; (ii) absence of neuropathological signs consistent with virus-induced CNS damage; (iii) absence of virus dissemination from the brain or viremia; (iv) absence of extraneural replication; (v) absence of shedding with saliva, urine or stool; (vi) presence of a neutralizing antibody response.

7.2.6 Clinical Experience

The first in human oncolytic PV, PVSRIPO, therapy was initiated at the PRTBTC at Duke University Medical Center in early 2012 as a Phase I trial in patients with histologically confirmed recurrent WHO grade IV malignant glioma (glioblastoma or gliosarcoma) (size of 1-5.5 cm). Key inclusion criteria in this ongoing clinical trial include: ≥ 18 years, adequate performance status/organ function, and prior PV immunity. Key exclusion criteria include: pregnant/breast-feeding females, those who have received radiotherapy less than 12 weeks prior, and those with known immune dysfunction or febrile and/or other systemic illnesses. Patients are given a booster of inactivated PV vaccine > 1 week prior to PVSRIPO infusion. Consented patients first undergo biopsy of the lesion to confirm recurrence and then have a catheter placed into the tumor ≥ 1 cm away from the ventricles. The catheter is tunneled 5 cm under the scalp and connected to an infusion pump. The agent is then infused at a rate of 0.5 mL/hour over a period of 6.5 hours. In the IND toxicology studies in non-human primates (NHP), no dose-limiting toxicities (DLTs) were observed and the MTD was not reached. The starting dose in this adult first-in-human trial was 1 x 10^8 TCID50, which is 1/10th of the highest non-toxic dose in NHPs from the definitive, IND-directed toxicology study and 1/50th of the highest non-toxic dose in NHPs from the dose-range finding toxicology study. In the dose escalation portion of the Phase I trial, cohorts of patients were dosed at 1 x 10^8 TCID50 (dose level 1), followed by 3.3 x 10^8 (level 2), 1 x 10^9 (level 3), 3.3 x 10^9 (level 4), and 1 x 10^{10} (level 5). The rationale and outcome associated with subsequent dose reductions to 3.3 x 10^8 (dose level 2), 5 x 10^7 (dose level -1), and 1.0 x 10^7 (dose level -2) are described below.

A total of 61 patients were treated on the Phase I study. The first 15 patients, who were treated at dose levels 1 through 5, constituted the dose escalation portion of the study. Due to observed tumor inflammation at the higher dose levels that could potentially have been related to treatment or tumor growth, the dose was de-escalated and six additional patients were treated at dose level 2. Due to the continuing requirement for steroids in all of the additional patients treated at dose level 2 because of tumor inflammation, due to treatment and/or tumor growth, a decision was made to de-escalate to dose level -1 (5 x 10^7 TCID50). Despite the fact that subjects on dose level -1 were able to remain off significant doses of steroids, it was believed that the subjects benefiting the most from PVSRIPO had been those who had experienced minimal inflammation.

As such, the dose of PVSRIPO was reduced to dose level -2 (1.0 x 10^7 TCID50) on 6/30/2016 with the goal of limiting the occurrence of undesirable burden from the inflammation and its treatment on as many subjects (including caregivers) as possible. Based upon additional animal studies, dose level -2 (1.0 x 10^7 TCID50) is considered to be a therapeutic dose. This dose reduction was not made due to concerns for the safety of the patients on dose level -1, but due to the hypothesis that dose level -2 would cause less inflammatory reaction to the virus inoculum and result in better survival and treatment response.

Based upon both pre-clinical tests highlighting the role of virus dose in PVSRIPO:host relations and clinical data collected thus far on subjects who were treated with either dose level -1 or dose level -2, the anticipated dose going forward is dose level -1. There does not appear to be evidence of pre-clinically or clinically important differences between the 2 dose levels, and most importantly no added benefit attributable to the reduced dose level -2. The need for initial treatment of cerebral inflammation either secondary to PVSRIPO or due to tumor growth, and the toxicity profile for the two dose levels, is comparable. As of 10/15/2019, eight patients

treated with PVSRIPO at any dose level remain alive with survival from initial PVSRIPO infusion ranging from 89 months to 30 months.

In June 2017, the Phase II study of PVSRIPO opened for patients diagnosed with recurrenty WHO Grade IV malignant glioma. Initially, this study was a single-site (Duke), randomized study that included 2 arms (PVSRIPO alone vs. PVSRIPO in combination with single dose of lomustine 8 weeks after PVSRIPO infusion). The lomustine arm was removed from the study in February 2019 after it was observed that patients randomized to receive lomustine often experienced a significant decline in blood counts, as expected. However, this could lead to the postponement of Avastin® (bevacizumab) therapy, often utilized for initial control of cerebral edema associated with the PVSRIPO anti-tumor response. The study is now a single arm, non-randomized study. In addition, the study was amended to add additional sites in October 2017. The study is remains open to enrollment at Duke, as well as at five other sites. As of 10/25/2019, a total of 89 patients have been treated on study across 4 centers.

7.3 Study Purpose/Rationale

Outcome for children with recurrent malignant glioma, especially GBM, is dismal with currently available therapeutic modalities. The blood-tumor barrier is a major impediment to therapy and approaches that bypass this barrier may be required. Oncolytic immunotherapy is a promising biologic approach to treatment that not only induces viral mediated tumor destruction, but also harnesses a complex immune response that can serve in disease control. The genetically engineered oncolytic PV (PVSRIPO) has tropism for tumor cells by virtue of expression of CD155 in tumor cells. To facilitate concentration of the therapeutic agent at the tumor site, while minimizing systemic exposure, PVSRIPO will be delivered directly into the tumor. Significant pre-clinical anti-tumor activity of PVSRIPO has been observed in several rodent tumor models and *in vitro*. PVSRIPO was devoid of neuropathogenicity when injected into the thalami of non-human primates even at doses as high as 5 x 10^9 TCID. Also, the phase I trial of PVSRIPO in adults with recurrent WHO grade IV malignant glioma still in the follow-up phase did not yield evidence of viral encephalomyelitis, poliomyelitis or meningitis with a proportion of patients enjoying prolonged disease survival up to 58+ months. The main toxicity attributable to PVSRIPO has been post-treatment peri-tumoral inflammation that has required prolonged steroid therapy and/or low dose bevacizumab to control edema. CD155 expression in pediatric GBM has been confirmed in 10 tumor samples. Therefore, a phase Ib study should be conducted in this high-risk pediatric population to evaluate feasibility, safety, and preliminary evidence of efficacy of the selected adult dose (5 x 10^7 TCID).

The Food and Drug Administration (FDA) recommended that the pediatric trial initially enroll patients 12 years of age and older and up to and including 18 years of age. We are including a number of WHO grade III and IV malignant brain tumor histologies and tumor locations in this trial. Specifically, eligible patients may include patients with recurrent WHO Grade III gliomas, such as anaplastic astrocytoma, anaplastic oligoastrocytoma, anaplastic oligodendroglioma, anaplastic pleomorphic xanthoastrocytoma, or ependymoma, as well as patients with WHO Grade IV gliomas, including glioblastoma or gliosarcoma. CD155 is avidly expressed in medulloblastoma, atypical teratoid rhabdoid tumor (ATRT), and embryonal tumors [26], so these histologies will be included here as well.

Given that the incidence of embryonal tumors, ependymoma, and glioma peaks at ages lower than the initial eligible age for this clinical trial (≥ 12 years old), the study has experienced relative challenges to enrollment during its first two years. The incidence of the aforementioned tumor types is higher in younger children approximately 5 to 9 years of age and then continuously falls after 9 years old to a nadir in early adulthood [27,28] In addition, the biology of brains in children aged 4 to 11 and the biology of the predominant types of brain tumors seen in this age range (i.e., glioma, ependymoma, and medulloblastoma) are similar to that of children who are 12 and over [1]. Tumors in children younger than 4 years old tend to have different genotypes and thus behave differently than tumors in children older than 4 years old [1]. There are currently active pediatric clinical trials in the US investigating the use of different oncolytic viruses, such as herpes simplex virus-1 (HSV-1) (NCT03911388 and NCT02451845) and a modified measles virus (NCT02962167), in a similar pediatric population. These trials also deliver the oncolytic virus via intratumoral administration and

include a population of children ranging in age from 3 to 18 years old in the case of aforementioned HSV-1 trial and children and adults ranging in age from 1 to 39 years old in the case of the modified measles virus.

As of June 26, 2019, five patients consented to participate in the study with three patients receiving PVSRIPO (i.e., 2 patients failed screening). Of the three treated patients, one was 9 years and treated under a planned deviation; one was 14 years old; and, one was 19 years old. Serious adverse events in these three patients that were considered possibly related to PVSRIPO include Grade 3 headache in two of the patients. Other toxicities considered possibly, probably, or definitely related to PVSRIPO have included Grade 1 eye disorders (intermittent double vision and difficulty seeing right-sided peripheral vision) (N=1), Grade 1 gastrointestinal disorders (nausea and vomiting) (N=1), Grade 1 general disorders (edema face) (N=1), Grade 1 general disorders (fatigue) (N=2), Grade 1 metabolism/nutrition disorders (anorexia) (N=1), Grade 1 metabolism/nutrition disorders (night sweats) (N=1), Grade 1 nervous system disorders (memory impairment) (N=1), and Grade 1 nervous system disorders (difficulty comprehending) (N=1). One of the three patients discontinued the study due to a secondary malignancy resulting from prior chemotherapy, while another patient discontinued due to disease progression; both of these patients are now deceased. One of the three patients discontinued the study due to re-resection of tumor and was being followed for survival as of June 26, 2019.

It is therefore reasonable to consider expanding enrollment of the clinical trial of oncolytic PV to younger children who have been diagnosed with a recurrence of eligible tumors who are between the ages of 4 and 12 years old. Accrual of all pediatric patients to this study was staggered so that no more than one patient was treated every 14 days for the first 5 patients enrolled. Staggered entry to this study was intended to minimize the number of pediatric patients exposed to PVSRIPO before adequate information about the sequelae of the inflammatory process within pediatric patients is known. Given limited enrollment over two years, the experience of treating 3 patients on study who range from 9 to 19 years of age, and the toxicity profile of PVSRIPO treatment in these patients, we believe expansion of the study to children as young as 4 years old will be safe and tolerable and will improve the ability to enroll on this pediatric study for patients diagnosed with recurrent supratentorial WHO Grade III malignant glioma or WHO Grade IV malignant glioma.

8 OBJECTIVES AND ENDPOINTS

Table 1. Objectives and Endpoints

	Objective	Endpoint	Analysis
Primary	Confirm the safety of the selected dose of PVSRIPO when delivered intracerebrally by convection-enhanced delivery (CED) in children with recurrent Grade III or IV MG	Percentage of patients that experience unacceptable toxicity during the 14-day period post-PVSRIPO treatment. Proportion of patients who require low-dose bevacizumab or steroid treatment due to an inflammatory reaction secondary to an immune response to PVSRIPO treatment will be calculated.	See Section 15.4
Secondary	Estimate Overall Survival (OS) in children with recurrent WHO Grade III and IV malignant glioma treated with a single dose of PVSRIPO	Proportion of patients alive 2 years after PVSRIPO treatment	See Section 15.5

Exploratory	Describe changes visualized on imaging due to intratumoral inoculation of PVSRIPO	Description of imaging changes	See Section 15.6
Other Exploratory	Describe changes in tumor volume visualized on imaging after intratumoral inoculation of PVSRIPO	Change from baseline in tumor volume based on imaging	See Section 15.7
Other Exploratory	Assess immunologic responses in peripheral blood and in serum	Change from baseline in immune function	See Section 15.7
Other Exploratory	Identify genetic predictors of response or failure of response to treatment with PVSRIPO	Identification of genetic markers as predictors of response	See Section 15.7

9 INVESTIGATIONAL PLAN

9.1 Study Design

9.1.1 Catheter Implantation

Systemic delivery of high molecular weight therapeutic agents to brain tumors is limited by the blood-brain barrier and increased interstitial pressure within the tumor. PV CNS invasion after intravenous administration via trans-endothelial passage is inefficient [29]. Intratumoral delivery bypasses these physiologic barriers and concentrates the therapeutic agent at the tumor site while minimizing systemic exposure. Therefore, PVSRIPO will be delivered directly into the tumor. A stereotactic biopsy will be performed prior to virus administration for frozen section confirmation of viable tumor and further analysis (see Section 9.1.4 below). The biopsy needle will be placed with stereotactic guidance by a Cosman-Robert-Wells, MRI-compatible, stereotactic head frame or a similar frameless device. Collection of biopsy tissue for pathologic diagnosis will be performed under traditionally accepted conditions according to standard of care. Up to three additional core biopsies will be obtained for molecular genetic testing as described in section 9.1.4.

If this biopsy does not confirm recurrent tumor, the subject will be withdrawn from the study.

Immediately following the stereotactically-guided tumor biopsy, a catheter [Vygon PIC-030 (Sophysa, Inc.; Crown Point, IN)] will be implanted in the operating room at a site the same or different from that used for the biopsy using sterile techniques under general anesthesia. Implantation will occur at a coordinate selected by the operating surgeon with the assistance of the clinically-approved iPlan Flow (BrainLAB). The catheter will be implanted at least 1 cm away from the ventricles. Based on our experience, a tumor ≤ 1cm from the ventricles can safely and feasibly have a catheter placed ≥ 1cm from the ventricles while minimizing the possibility of infusate entering the ventricles. The catheter will be tunneled beneath the scalp for a distance of at least 5 cm to aid in the prevention of infection. A CT scan may be used to confirm catheter placement post-operatively.

9.1.2 Agent Infusion

Refer to the study Investigational Product Handling Plan (IPHP) for additional details regarding the infusate preparation and infusion procedure. The entire volume of the prepared infusate will be provided in a sterile infusion syringe by the investigational pharmacist and connected to the catheter under sterile conditions in the Pediatric Intensive Care Unit (PICU) just prior to beginning of infusion. Due to the complexity of scheduling all of the necessary components for the infusion (operating room time, pharmacy time, and radiology appointments), a +1 day window has been built into the study for the study drug infusion. This means that the infusion is allowed to start the following day after the

biopsy/catheter placement. This will still be considered “day 0” with regard to the protocol and the timing of the subsequent events. At the time of virus injection, emergency drugs, including epinephrine and diphenhydramine will be available and the neurologic status, oxygen saturation, and cardiac rhythm will be monitored. Drug infusion will occur in the PICU so that all other emergency facilities will be available. Patients will be treated with a prophylactic antibiotic prior to biopsy and catheter insertion per DUHS neurosurgical standard practice.

Based on our own experience, previously published reports [5] and IRB- and FDA-approved trials using similar infusion techniques, patients will be infused at a rate of 0.5mL/hr. A Medfusion 3500 or 3010a infusion pump will be pre-programmed to a delivery rate of 500 µL/hr. The infusate will be loaded in a syringe into the syringe pump at the initial onset to avoid any interruptions in the infusion. The total amount of the infusate delivered to the patient will be 3 mL. The infusion pump will be programmed for delivery of the 3.0mL at a rate of 0.5ml/hr, over approximately 6.5 hours. The catheter itself (30 cm length, 1 mm interior diameter) cannot be preloaded with virus suspension. To account for this catheter dead space, the pump will be stopped when the delivered amount is between 3.125 to 3.250 mL. The infusion will be performed using a Medfusion 3500 or 3010a (Smiths Medical ASK, Inc., Minneapolis, MN) syringe infusion pump. The catheter will be removed in the PICU following the post-PVSRIPO infusion MRI. No sedation is required for catheter removal.

The infusion catheter (PIC 030) and infusion tubing (PIT 400) are manufactured by Sophysa, Inc. (Crown Point, IN). The Infusion Catheter Kit is a 30 cm clear, open-ended catheter (1.0 mm ID/2.0 mm OD) with 1 cm markings for 20 cm. The catheter comes with a 30 cm stainless steel stylet, a barbed female luer lock with cap and a stainless steel trocar. The Infusion Tubing Kit consists of a 3-way stopcock connector with air filter, 4 m of microbore tubing with antisiphon valve, a red, vented cap and a white luer lock cap. The catheter products are packaged sterile and non-pyrogenic and are intended for single (one-time) use only.

Acute Reaction. Any acute reaction symptoms determined to be an acute reaction to the study drug will be managed by the PICU and pediatric neurosurgical teams.

9.1.3 Gadolinium Distribution Quantitation (applies only to patients treated prior to October 31, 2019)

This gadolinium tracking MRI procedure has been removed and applies only to patients treated prior to October 31, 2019. Magnevist® is no longer being manufactured and, as such, is no longer being included in the infusate.

MRI imaging, within 4 hours of the completion of infusion, was registered to define the shape and position of the contrast agent distribution relative to the patient’s brain anatomy in patients treated prior to October 31, 2019. Because enrollment criteria for these patients was limited to patients aged ≥ 12 years old, sedation was not required for the MRI. While gadolinium–diethylene triamine pentaacetic acid (Gd-DTPA) is a widely available MRI contrast agent, there has been speculation that its small molecular weight (938 Dalton) could limit its ability to predict the distribution of the larger molecules typically infused therapeutically with CED [reviewed in Reardon et al., 2011 [30]]. Development of large molecule tracers labeled with gadolinium (Gd) has been problematic, but it has been stipulated that infusion of low molecular weight Gd-DTPA can predict the distribution of larger molecules by systematic post-infusion manipulation of the images based on theoretical differences in the predicted distribution of the Gd-DTPA and the therapeutic drug being infused [31]. This has been confirmed by simultaneously infusing a patient with a supratentorial recurrent malignant glioma with an epidermal growth factor receptor (variant III) (EGFRvIII)-targeted immunotoxin in combination with ^{124}I-human serum albumin (HSA) [to permit positron emission tomography (PET) imaging] and Gd-DTPA. Gd-DTPA co-infusion provided direct information about the distribution of large molecules with high resolution [31]. In combination with fluid-attenuated inversion recovery (FLAIR) imaging, Gd-DTPA co-infusion provides additional information about leak into cerebrospinal fluid spaces and resection cavities [32].

9.1.4 Biopsy Sampling and Analyses

Biopsy material will be obtained from tumor tissue prior to virus administration. This tissue material will be subjected to routine histology to confirm tumor recurrence by the study neuropathologist, Dr. R. McLendon or his designate.

Molecular genetic tests will also be conducted on extracts of tumor cells from the protocol-specified biopsy prior to PVSRIPO infusion. After acquiring sufficient tissue for standard clinical pathologic testing, up to three additional core biopsies will be obtained, if possible. These additional core biopsies will be frozen in optimal cutting temperature (OCT) fixative and kept at liquid nitrogen temperature. They will be used for genetic analysis, including full genome or full exome sequencing as well as other molecular genetic testing. The goal of these molecular genetic tests is to identify genetic predictors of response or failure of response to treatment with PVSRIPO. The molecular genetic testing will include, but is not limited to, DNA sequencing, gene amplification, and gene expression.

The goal of these molecular genetic tests is to identify genetic predictors of response or failure of response to treatment with PVSRIPO. The amount of tissue required for this testing should be sufficient to yield a minimum of one microgram of DNA.

Following PVSRIPO administration and outside the context of this protocol, patients may undergo tumor resection or biopsy as medically indicated for reasons not part of the investigative protocol. Should subjects have a resection at a later time, we will request samples of this resected tissue for tissue analysis. If subjects agree to this by opting in on the informed consent, portions of resected tissue will be delivered to the study neuropathologist, Dr. R. McLendon or his designate, for histopathological analyses and to Dr. M. Gromeier or his designate for correlative molecular analyses. Tissue will be processed and stored in the Brain Tumor Biorepository at Duke (Duke IRB Pro0007434). Samples are assigned a patient de-identified biorepository number. The Biorepository database is Duke IRB-approved, accessible by Biorepository personnel only, and includes relevant data associated with the sample, such as Biorepostiory number, treatment with PVSRIPO, dates related to the sample, diagnosis, age, race, gender, pathology, and any other relevant information. Samples from tissue collected and stored for the aforementioned purpose may be analyzed within Duke at the request of the study investigators or may be sent to outside collaborators for analysis. Samples will always be identified by Biorepository number and/or Study ID number. Investigators will submit any such requests to the Biorepository using their internal tissue request form. Additional pathology analyses may include, but is not limited to, EGFRvIII and EGFRwt status, TERT, IDH 1 and 2, and MGMT IHC and MGMT promoter methylation, if a sufficient amount of tissue remains after standard clinical pathologic testing.

Tissue that is processed and stored by the Brain Tumor Biorepository for this study will be maintained indefinitely in the Biorepository for future testing until such time as the tissue supply designated for research is exhausted.

9.1.5 Definition of Unacceptable Toxicity

Toxicities will be graded according to the NCI CTCAE version 4 criteria. Any Grade 3 or any Grade 4 toxicity that is not reversible within 2 weeks, any life-threatening event deemed related to the study drug, or any treatment-related death will be considered unacceptable.

Any Grade 2 or higher autoimmune toxicities, particularly those affecting vital organs (e.g. cardiac, renal, CNS), will be considered an unacceptable toxicity, if it occurs within 2 weeks post-infusion. Grade 3 or higher surgical complications from insertion of the catheter are considered an unacceptable toxicity.

Exceptions to these unacceptable toxicities are as follows:

- Events associated with the biopsy procedure/catheter placement: Seizures or hemorrhages occurring during anesthesia or the biopsy/catheter insertion proper prior to administration of the agent if Grade 2 or lower.

- Seizures: Due to the nature of the disease under investigation in this protocol, patients may have pre-existing seizures or be susceptible to new seizures as a result of the underlying disease process. Although seizures may be defined as Grade 3 or 4 toxicities under NCI CTC, and will be reported as such in this protocol, seizures will not be considered an unacceptable toxicity if, in the opinion of the site Principal Investigator they have not increased in frequency or can be attributed to another recognized cause of increasing seizure frequency such as sub-therapeutic anti-convulsant levels or biopsy proven tumor progression.

- New or worsening neurologic deficits: Due to the nature of the disease under investigation in this protocol, patients may have an increase in pre-existing neurologic deficits or may develop new neurologic deficits as a result of tumor invasion. A new neurologic deficit, which resolves within 2 weeks after initiation of medical therapy, will not be considered an unacceptable toxicity. New neurological symptoms will not be an unacceptable toxicity if they can be ascribed to tumor progression (e.g. documented with histopathologic analyses of biopsy tissue), or they respond to treatment (e.g. oral steroids) within 2 weeks.

- Thromboembolism: While deep vein thrombosis (DVT) is not as common in children as in adults with malignant glioma, patients may have undiagnosed pre-existing DVTs or be susceptible to the development of DVTs due to the underlying disease process. Although DVT may be defined as Grade 3 or 4 toxicities under NCI CTC, and will be reported as such in this protocol, DVT will not be considered an unacceptable toxicity in this protocol.

- Syndrome of Inappropriate Antidiuretic Hormone (SIADH): Due to the high incidence of SIADH in this patient population, patients may be susceptible to the development of SIADH due to the underlying disease process. Although SIADH may be defined as Grade 3 toxicity under NCI CTC, and will be reported as such in this protocol, SIADH will not be considered an unacceptable toxicity in this protocol unless it is refractory to medical management.

- Muscle Weakness and Weight Gain: Due to the high incidence of muscle weakness and weight gain in patients taking steroids in this patient population, patients may be susceptible to the development of muscle weakness or weight gain which is due to steroids alone. Although muscle weakness may be defined as Grade 3 or 4 toxicity and weight gain $\geq$ 20% may be defined as Grade 3 toxicity under NCI CTC, and will be reported as such in this protocol, muscle weakness or weight gain will not be considered an unacceptable toxicity in this protocol if the patient has required steroids greater than physiologic doses in the interval between the immunization and the development of the toxicity.

9.1.6 Safety Considerations

- Surgical Complications: The stereotactic tumor biopsy and catheter implantation procedure carries a risk for cerebrospinal fluid leak, infection, hemorrhage, loss of neurologic function, non-neurologic complications and death. These risks depend primarily on the preoperative condition of the patient, the size and location of the tumor and associated diseases. The potential risk for the patient will be discussed in detail with the patient and family.

- Anesthesia: Patients undergoing general anesthesia may be subjected to associated risks including pneumothorax, pneumonia, airway injury, hypotension, myocardial infarction, stroke, hepatic and renal injury and death.

- Poliomyelitis: PVSRIPO has been tested in non-human primates according to the WHO standardized monkey neurovirulence tests. These tests revealed the absence of neuropathogenic properties, evident as failure to induce symptoms of poliomyelitis in non-human primates after intracerebral inoculation of virus. However, PVSRIPO is a replication-competent viral agent that, in principle, retains the potential to cause motor neuron damage. Possible complications include transient or permanent mono- or paraparesis, paralysis, and life-threatening respiratory insufficiency.

- Virus Recombination and Mutation: PVSRIPO retains the ability to alter its genome during replication upon administration to patients. Various mechanisms are known to lead to genetic adaptation, including spontaneous mutagenesis and recombination that may give rise to viral progeny with changed properties. Empirical studies in tissue culture and laboratory animals demonstrated that prolonged passaging in GBM cells does not select for genetic changes in viral progeny. However, the occurrence of such events cannot be categorically excluded in patients receiving intracerebral PVSRIPO. Genetic changes may cause an altered phenotype of PVSRIPO, including adaptation to improved virus replication in the normal CNS.

- Long-Term Sequelae of Virus Injection: PVSRIPO does not encode foreign genetic material; PVs are unable to insert viral genetic material in the host genome and PVs are incapable of inducing latent or chronic CNS infection. Therefore, PVSRIPO is unable to induce long-term expression of foreign polypeptides or long-term persistence. Thus, there are no long-term neurologic consequences to intracerebral PVSRIPO administration in study subjects. For these reasons, no specific measures to analyze the potential for persistence of virus replication in the CNS of long-term survivors are indicated.

- Gastrointestinal Infection and Virus Excretion: After oral uptake, PV replicates in the gastrointestinal tract and is excreted by infected individuals' stool. Gastrointestinal viral replication usually is asymptomatic, but may cause mild symptoms of gastrointestinal discomfort. Tests of PVSRIPO in non-human primates and in subjects from the phase I study suggest that excretion of PVSRIPO does not occur after intracerebral inoculation in immunized/boosted patients. This is based on thorough testing in patients in our Phase 1 clinical trial and much historical evidence from investigations in non-human primates.

- Cerebral Edema and Increased Intracranial Pressure: Cerebral edema may be secondary to the disease process itself, the surgical procedure, necrosis from previous radiation, or inflammation due to immune infiltration of the brain or destruction of tumor cells. Symptoms may include, but are not limited to, severe headache, confusion, lethargy, unresponsiveness, coma, or focal neurological deficits. Patients will be monitored throughout the course of the study and upon any signs or symptoms of cerebral edema, may have their steroid doses increased or receive treatment with an osmotic diuretic, or surgical decompression. Edema that fails to respond to aggressive therapy may lead to permanent neurological impairment. The probability of this risk can be predicted to some degree based upon tumor size, location, pre-operative neurological impairment, and post-operative course prior to virus administration.

  Special Consideration: In the event that a patient demonstrates neurologic or radiographic signs suggestive of an inflammatory reaction secondary to the immune response triggered by PVSRIPO that requires a dose of dexamethasone above 4 mg a day after the first 2 weeks post PVSRIPO infusion, dexamethasone will not be increased any further. Instead, patients will be treated with bevacizumab 7.5 mg/kg IV every 3 weeks and neuroimaging (MRI) after 3 doses (every 9 weeks +/- 2 weeks) to assess if further treatment with bevacizumab is needed to control the cerebral inflammation. Bevacizumab will not be provided by the study. Every attempt should be made to discontinue dexamethasone. Once patients start bevacizumab, the follow-up visit schedule will be adjusted to coincide with the timing of the bevacizumab instead of the prior PVSRIPO infusion. Therefore, patients will return approximately every 9 weeks for an evaluation with MRI.

Per the packet insert for bevacizumab dated 12/2017, the safety and effectiveness of bevacizumab have not been established in the pediatric population [33]. Bevacizumab is currently not approved by the FDA for use in patients under the age of 18 years. "In published literature reports, cases of non-mandibular osteonecrosis have been observed in patients under the age of 18 years who have received Avastin [33]." In initial Phase I and II clinical trials, four potential bevacizumab-associated safety signals were identified: hypertension, proteinuria, thromboembolic events, and hemorrhage. Additional completed Phase II and Phase III studies of bevacizumab in adults, as well as spontaneous reports have further defined the safety profile of this agent. Bevacizumab-associated adverse events identified in Phase III trials in adults include congestive heart failure (CHF) primarily in metastatic breast cancer, gastrointestinal perforations, wound-healing complications, and arterial thromboembolic events (ATEs). These and other safety signals are described in further detail in the bevacizumab package insert.

If there are adverse events or other circumstances prohibiting the use of bevacizumab, we will use corticosteroids or surgery, or other interventions deemed more appropriate for the patient by the treating physician, if needed, to treat the inflammatory reaction secondary to PVSRIPO.

- Risk of Infection: The intracerebral catheter placement and infusion may include the risk of infection. However, similar procedures including stereotactic biopsy and ventriculostomy placement are commonly used in the routine clinical care of patients with malignant brain tumors with a very low and acceptable rate of infection. In the most extreme situation, however, infection may lead to systemic bacterial/fungal sepsis and possibly death. The risk of infection will be minimized though by performing catheter implantations in the Operating Room. Patients will be monitored throughout the course of the study for any signs and symptoms of infection. If an active infection is suspected, patients will be cultured and treated with appropriate antibiotics.

- Risk of Phlebotomy: Drawing blood or inserting an intravenous catheter into an arm vein may result in bruising or swelling in the area of the insertion, bleeding at the site of the needle puncture, light headedness, fainting and very rarely, local infection, which may be severe. These risks are reduced by the fact that the blood will be drawn by a qualified physician, nurse or phlebotomist (a professional trained to draw blood).

- Risks of MRIs: Risks and/or discomforts associated with MRI scans include anxiety produced from being in a tight, enclosed space (claustrophobia). In addition, the machine operates using a large and powerful magnet, which attracts certain metals. Therefore, people with these metals in their bodies (specifically pacemakers, infusion pumps, metal aneurysm clips, metal prostheses, joints, rods or plates) will be excluded from the study. Patients will also be checked to make sure that they do not bring any metal objects into the MRI facility. Dental fillings are less affected by the magnetic fields generated and are therefore permitted. It will be asked that patients let the physicians conducting this study know of any metal in their bodies other than dental fillings. Depending upon the age of the patient, sedation for imaging may be needed. Risks of sedation include an allergic reaction, aspiration, and over-sedation. Use of an IV may cause bruising. Occasionally, an infection develops at the IV site.

- Allergic Reactions to Contrast Agents: During the MRI, patients will be given a contrast agent. The agent is given routinely to obtain enhanced MRI scans of the brain. The agent is administered through the vein and requires the placement of an IV catheter. The catheter placement is similar to drawing blood except that the catheter remains in the vein during the time the agent is actively delivered. The risks of a blood draw and insertion of a catheter are similar. There have been a few, rare cases of allergies to the agent used in MRI contrast enhanced scans. Patients with any known severe allergies (anaphylaxis) to contrast agents will be excluded from the study. Patients with mild allergies (i.e., rash only) will be pretreated

with Tylenol and Benadryl prior to injection of the contrast agent. In addition to the gadolinium (contrast agent) being given for MRI contrast, it will also be co-infused with the study drug to assess infusion distribution. It is not FDA approved for gadolinium to be administered in this way. The potential risks of intracerebral infusion of gadolinium contrast agents are not completely known but are believed to be small. Risks for infusion of gadolinium contrast agents intrathecally for procedures such as cistemography have been somewhat better studied, and were recently summarized by Selcuk et al (2009) [34]. Encephalopathy, coma and seizures have been reported as side effects in case reports of accidental administration of large amounts of gadolinium contrast agents intrathecally in humans [32,35]. When these contrast agents are used in appropriately low dose, however, the risk of intrathecal administration appears reasonably low. No neurological sequelae attributable to the procedure were detected in a series of 85 patients [34], in another series of 95 patients at a one year follow-up [36], or in any of 51 patients after over 4 years of mean follow-up in another study [37]. Although these results cannot be extrapolated to the procedure proposed in the current research, they indicate that direct exposure of the brain to small amounts of gadolinium contrast agent is generally well tolerated.

A rare but serious adverse reaction has been observed in patients that received a gadolinium-based contrast material during MRI examinations, a reaction called nephrogenic systemic fibrosis (NSF). Patients with kidney disease are at increased risk of developing NSF. NSF may cause skin thickening, joint pain and/or swelling. In rare cases NSF can lead to lung and heart problems and cause death.

- Risks to Household Contacts of Study Subjects: Primate toxicology studies showed that intracerebral infusion of PVSRIPO does not lead to extraneural dissemination or replication and, hence, is not associated with virus shedding. Therefore, and because PV transmission occurs via the fecal-oral route, the likelihood of unintended exposure of patient household contacts is exceedingly low. If accidental exposure occurred, it would equal the risk of exposure to any type 1 Sabin vaccine virus or vaccine virus derivatives. Thus, in essence, exposure with PVSRIPO is equal to oral immunization with a safe version of type 1 Sabin. Since type 1 Sabin vaccine virus or vaccine virus derivatives have to be considered part of the human environment, exposure to PVSRIPO would not represent an added risk beyond the possibility for exposure that already exists.

- Unknown Risks: The overall risk classification of this research is unknown.

9.1.7 Missed Doses

This is not applicable, as the PVSRIPO is a single administration agent.

9.1.8 Concomitant Medications

9.1.8.1 Steroids

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

9.1.8.2 Anticonvulsants

Anticonvulsants drugs should be used or continued, if indicated. Use of such anticonvulsants should be recorded in the electronic database.

9.1.8.3 Growth Factors

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

9.1.8.4 Anti-emetics
The use of anti-emetics will be at the investigator's discretion. Use of anti-emetics should be recorded in the electronic database.

9.1.8.5 Proton Pump Inhibitors
The use of proton pump inhibitors (PPI) (e.g. rabeprazole, omeprazole, pantoprazole, lansoprazole or esomeprazole) is allowed on this study.

9.1.8.6 Febrile Neutropenia
Febrile neutropenia should be managed according to the local institutional guidelines. Measures include laboratory testing, blood and urine cultures, and institution of broad spectrum antibiotics.

9.1.8.7 *Pneumocystis jiroveci* pneumonia (PJP) prophylaxis
The use of medication (i.e., Bactrim) for PJP prophylaxis in patients on chronic steroids is recommended, but is at the investigator's discretion.

9.1.8.8 Neurosurgical Procedures
If a neurosurgical procedure is required for a reason other than tumor progression (i.e. the onset of hydrocephalus), these procedures should be documented, but will not constitute criteria for declaring the patient "off therapy."

9.1.9 Study Drug Blinding
Not applicable

9.2 Rationale for Selection of Dose, Regimen, and Treatment Duration
Dose: The dose for this trial was selected based on IND-directed toxicity studies [25] and on experience from an ongoing study in adults with recurrent WHO grade IV malignant glioma. Dose-range finding and toxicity studies in non-human primates documented the absence of viral encephalomyelitis, poliomyelitis and meningitis with intracerebral injection of PVSRIPO up to a dose of 5 x 10^9 TCID50 [25]. In the adult WHO grade IV malignant glioma study, 4 patients were dosed at the maximum dose of 1 x 10^{10} TCID50 without signs of viral encephalomyelitis, poliomyelitis or meningitis. Given that the brain size of the target population for this trial (children and young adults aged 4-21) is nearly identical to that of adults, and that the human brain is ~10-times the size of the primate species used in the IND-directed toxicity studies (*M. Fascicularis*), the dose of 5 x 10^7 TCID50 in this trial is 500-times below the maximum feasible safe dose in *M. Fascicularis* and 1000-times below the maximum dose in the adult WHO grade IV malignant glioma study. The proposed dose of 5 x 10^7 TCID50 corresponds to the dose selected for future studies based on the Phase I adult WHO grade IV malignant glioma study. If unacceptable toxicity that requires dose reduction occurs, according to Section 15.4.1, we may consider a PVSRIPO dose reduction to 3.3 x 10^6 TCID50.

Regimen and Treatment Duration: The treatment regimen for PVSRIPO is single intracerebral infusion, as in the ongoing adult WHO grade IV malignant glioma study.

9.3 Rationale for Correlative Studies
While tumor-selective PVSRIPO propagation is an important mediator of cytotoxicity, significant intra- and peritumoral inflammation likely ensues. This raises the possibility that an immunogenic response is being generated, certainly against the virus itself and with great likelihood against the tumor as well (**Figure 2**) [15]. While the exact host immune response to PVSRIPO oncolysis is currently unknown (but is being investigated), host innate antiviral defenses are likely to trigger a broad immune effector cascade that needs to be examined in patients receiving PVSRIPO therapy. Blood will therefore be collected for immune function studies before and at periodic intervals following treatment with PVSRIPO.

9.4 Definition of Evaluable Subjects

Any patient who has undergone surgery, placement of the catheter, and any dose of PVSRIPO will be evaluable for both toxicity and efficacy of agent.

9.5 Early Study Termination

This study can be terminated at any time for any reason by the PI-sponsor. If this occurs, all subjects on study should be notified as soon as possible. Additional procedures and/or follow up should occur in accordance with Section 12.6.2.

10 STUDY DRUG

10.1 Names, Classification, and Mechanism of Action

PVSRIPO is a modified version of the serotype 1 live-attenuated (Sabin) PV vaccine (PV1) and its immunogenic properties and potential for long-term sequelae are expected to be similar. PV1S has been safely administered to >10 billion individuals worldwide without untoward long-term sequelae. The only known effect of PV1S administration to human subjects is neutralizing immunity to PV.

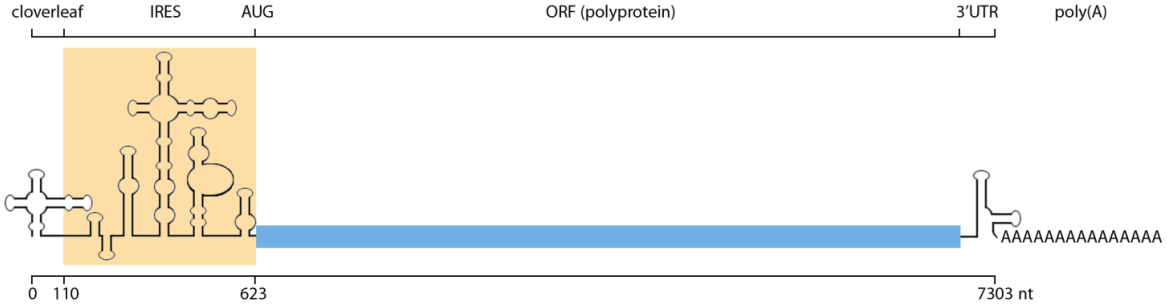


Figure 3. Genetic structure of PVSRIPO.

PVSRIPO is PV1S containing a heterologous IRES of HRV2 (**Figure 3**). The IRES is a *cis*-acting, non-coding genetic element within the 5' untranslated region (UTR) of all enteroviruses and is essential for translation of the viral genome. PVSRIPO is a non-enveloped, positive-sense ssRNA virus with a genome of ~7300 nucleotides (nt) in length (**Figure 3**). PVSRIPO particles consist of a proteinaceous capsid composed of 60 copies of each of 4 capsid proteins (VP1-VP4) arranged in icosahedral geometry. Since the coding regions for the viral polyprotein (giving rise to all viral polypeptides) of PVSRIPO and PV1S are the same, the physical structure of the viral capsid and all non-structural viral polypeptides of PVSRIPO and PV1S are identical.

10.2 Packaging and Labeling

PVSRIPO is formulated in 50 mM sodium phosphate in 0.9% sodium chloride, pH 7.4 with 0.2% human serum albumin. It is provided in sterile, single use containers. PVSRIPO was manufactured at the Biopharmaceutical Development Program/SAIC-Frederick at NCI-Frederick. Prior to October 31, 2019, for tracking intratumoral/intracerebral distribution of the inoculum, the study agent suspension was supplemented with gadolinium-DTPA diamide (Magnevist®) at a final concentration of 1 mM and Magnevist® was stored at room temperature. Magnevist® is no longer being manufactured in the US so has been removed from the infusate along with the corresponding post-infusion MRI within 4h of infusion.

10.3 Supply, Receipt, and Storage

Refer to the study Investigational Product Handling Plan (IPHP) for details regarding supply, receipt and storage of study materials. The study agent and vehicle will be supplied directly to Investigational Chemotherapy Service (ICS) by the Sponsor (or designee). The study agent will be shipped via approved methods in the appropriate packaging on dry ice.

PVSRIPO should be stored long-term in an ultra-low temperature freezer (e.g., a ≤ -80°C freezer). Thawed vials should be kept at 4°C whenever possible. Thawed vials of PVSRIPO are stable at 4°C for 48 hours. Once thawed, it is a clear colorless liquid with no evidence of particulates or foreign matter.

10.4 Dispensing and Preparation

Refer to the study IPHP for details of PVSRIPO dispensing and preparation. In brief, preparation of PVSRIPO will occur in a biosafety cabinet designated for viral vector agents. For thawing, vials containing PVSRIPO should be removed from the -80°C freezer and kept at room temperature. PVSRIPO contained in the clinically intended delivery apparatus is stable at room temperature for 18 hours, but should be used as soon as possible. The vials contain a clear, aqueous solution, which does not require reconstitution. **Do not thaw with heating devices (waterbath, heat-block) as a loss of potency may result. Do not expose vials to UV-light (in biosafety cabinets), as a loss of potency will result**. Thawed vials should not be re-frozen/thawed for later use, because potency may be lost. Thawed vials/study syringes containing PVSRIPO, which were kept for >48 hrs at 4°C prior to the infusion procedure, should be discarded, as a loss of potency may have occurred. Thawed vials/study syringes containing PVSRIPO, which were exposed for >12 hrs at room temperature prior to the infusion procedure, shall be discarded as a loss of potency may have occurred. For aliquot preparation, the agent will be thawed slowly on ice (~4°C) and kept at that temperature. The vials take approximately 30 minutes to thaw. The manufacturer will provide the study agent's potency (as tissue culture infectious doses) and will also supply the appropriate vehicle for aliquot preparation. Aliquot preparation will occur in the ICS. All handling of the study agent will occur in a biosafety cabinet or a similarly contained environment.

Any materials in contact with the study agent, e.g. syringes, vials, etc., will be disposed of as biological waste. The final desired aliquot of the study agent infusate will be prepared at the intended volume sufficient for priming the infusion tubing and for infusion. . The capped infusion syringe containing infusate (and primed infusion tubing if primed in pharmacy) will be transported to the study site in a 'ziplock' bag placed in a portable cooler or similarly contained transport device with a frozen ice block/ice pack, in order to maintain a temperature of approximately 4°C. No need to monitor the temperature during transport to the bedside.

10.5 Compliance and Accountability

Drug accountability records will be maintained for all clinical trial supplies. All empty and partially used clinical trial supplies will be destroyed in accordance with institutional guidelines. ICS will maintain detailed documentation of the receipt and/or destruction of the study agent. All materials coming in contact with the study agent, the syringe delivered from ICS, tubing, dressings or coverings used to protect the trepanation site, will be disposed of as biological waste in the treatment room.

10.6 Disposal and Destruction

All surgical materials used in the procedure and (potentially) coming in contact with the study agent will be disposable. These materials will be disposed of as biological waste using established procedures. Used sharps will be disposed in biohazard sharps container and incinerated for final disposal.

11 SUBJECT ELIGIBILITY

11.1 Inclusion Criteria

1. Patients must have a recurrent supratentorial WHO Grade III malignant glioma (anaplastic astrocytoma, anaplastic oligoastrocytoma, anaplastic oligodendroglioma, anaplastic pleomorphic xanthoastrocytoma, ependymoma) or WHO Grade IV malignant glioma, medulloblastoma, or atypical teratoid/rhabdoid tumor (ATRT) based on imaging studies with measurable disease (≥ 1 cm and ≤ 5.5 cm).

a. The prior histopathology must be consistent with a World Health Organization (WHO) Grade III or IV malignant tumor confirmed by the study pathologist, Roger McLendon, or his designee.
b. The **tip** of the infusion catheter can be placed ≥ 1cm from any ventricle.

2. There is no standard of care treatment for children with Grade III/IV gliomas; however, patients must have received some form of definitive treatment, i.e., standard therapy with known clinical benefit, for their initial diagnosis prior to their recurrence/progression. Definitive treatment includes maximal safe resection (if possible) and radiation therapy with or without chemotherapy. *(Please note that patients who are unable to receive radiation therapy due to genetic disorders that put them at significant risk for radiation-induced secondary malignancies (i.e., Gorlin's syndrome or NF1 mutation) are still eligible to participate)*.
3. All patients must be ≥ 4 years of age and ≤ 21 years of age at the time of entry into the study.
4. The patient must have a Lansky or Karnofsky Performance Score (KPS) of ≥ 70% at the time of entry.
5. Laboratory Studies:
    a. Platelet count ≥ 125,000/μl prior to biopsy. Platelets ≥ 100,000/μl prior to infusion;
    b. Prothrombin and Activated Partial Thromboplastin Times ≤ 1.2 x upper limit of normal (ULN) prior to biopsy;
    c. Positive serum anti-poliovirus titer ≥ 1:8 prior to biopsy;
    d. Creatinine ≤ 1.2 x ULN prior to biopsy;
    e. Total bilirubin, AST, ALT, alkaline phosphatase ≤ 2.5 x ULN prior to biopsy;
    f. Neutrophil count ≥ 1000 /μl prior to biopsy;
    g. Hemoglobin ≥ 9 gm/dl prior to biopsy (can be transfused).
6. The patient must have received a boost immunization with trivalent inactivated IPOL™ (Sanofi-Pasteur) ≥ 1 week prior to administration of the study agent.
7. At the time of biopsy, prior to administration of virus, the presence of recurrent tumor must be confirmed by histopathological analysis.
8. A signed informed consent form approved by the Duke University Institutional Review Board (IRB) will be required for patient enrollment into the study. Patients (if 18 years old) or their parent(s) or guardian(s) (if younger than 18 years old) must be able to read and understand the informed consent document and must sign the informed consent indicating that they are aware of the investigational nature of this study. Children who are ≥ 12 years old and < 18 years old should provide written assent to participate in the study. Children who are ≤ 6 years old and < 12 years should provide verbal assent to participate in the study.

11.2 Exclusion Criteria

1. Pregnant or breast-feeding. Female patients of child-bearing potential or female sexual partners (who are of child-bearing potential) of male patients must use at least one of the following methods of medically acceptable contraceptives: approved hormonal contraceptives (such as birth control pills, patches, implants or infusions), an intrauterine device (IUD), or a barrier method of contraception (such as a condom or diaphragm) used with spermicide. Because all patients are required to have a boost immunization of trivalent inactivated IPOL™, there should be no risk of transmission of a mother to her fetus after receiving intracranial PVSRIPO. As such, patients who become pregnant after receiving PVSRIPO will continue to be monitored in the same manner, i.e. per protocol, unless the assessment is contra-indicated during pregnancy. Partners who become pregnant will sign a Pregnant Partner Information Form and information regarding the pregnancy and its outcome may be collected.
2. Patients with an impending, life-threatening cerebral herniation syndrome, based on the assessment of the study neurosurgeon.
3. Patients with an active infection requiring intravenous treatment or having an unexplained febrile illness (T_{max} > 99.5°F).

4. Patients with known immunosuppressive disease or known human immunodeficiency virus infection.
5. Patients with unstable or severe intercurrent medical conditions such as severe heart (New York Heart Association Class 3 or 4) or lung (FEV_1 < 50%) disease, uncontrolled diabetes mellitus.
6. Patients with allergy to human serum albumin.
7. Patients with a known severe allergy (anaphylaxis) to gadolinium. Patients with mild allergies (e.g., rash only) are eligible and may be pretreated per institutional guidelines prior to injection of the contrast agent.
8. Patients with a previous history of neurological complications due to PV infection..
9. Patients who have not recovered from the toxic effects of prior chemo- and/or radiation therapy. Guidelines for this recovery period are dependent upon the specific therapeutic agent being used:
    a. Patients who are less than 12 weeks from radiation therapy, unless progressive disease outside of the radiation field or 2 consecutive scans with disease progression or histopathologic confirmation of recurrent tumor.
    b. Patients who have received chemotherapy or bevacizumab ≤ 4 weeks [except for nitrosourea (6 weeks) or metronomic dosed chemotherapy such as daily etoposide or cyclophosphamide (1 week)] prior to starting the study drug unless patients have recovered from side effects of such therapy.
    c. Patients who have received immunotherapy ≤ 4 weeks prior to starting the study drug unless patients have recovered from side effects of such therapy.
10. Patients with neoplastic lesions in the brainstem, cerebellum, spinal cord, intraventricular tumors, pituitary tumors, leptomeningeal disease, or other locations at the discretion of the treating neurosurgeon.
11. Patients with a diagnosis of agammaglobulinemia, that is:
    a. Undetectable anti-tetanus toxoid IgG
    b. Known history of agammaglobulinemia
12. Patients who are on dexamethasone receiving > 4 mg/day in the two weeks prior to admission for intra-cerebral delivery of PVSRIPO or who demonstrate worsening steroid myopathy.
13. Patients with prior, unrelated malignancy requiring current active treatment with the exception of cervical carcinoma *in situ* and adequately treated basal cell or squamous cell carcinoma of the skin.

12 SCREENING AND ON-STUDY TESTS AND PROCEDURES

Table 2. Schedule of Study Tests and Procedures

Description	Within 6 months	Screening: Within14 days prior to catheter placement	Screening: Within 5 days prior to PVSRIPO	Catheter Placement biopsy, PVSRIPO infusion	After Completion of Infusion	Post infusion (follow-up period) [a]					
Week						0	1	2	4	8	16, 24, 32, 40, 48
Day		Within 14 days prior to catheter placement	Within 5 days prior to PVSRIPO	0	0	1	7	14	28	56	112, 168, 224, 280, 336
General Evaluations											
Physical Exam		X	X			X[b]	X	X	X	X	X
Neurologic Exam		X	X			X[b]	X	X	X	X	X
Performance Status		X	X				X	X	X	X	X
Adverse Events[h]	X	X	X	Continuous							
Laboratory Evaluations											
Poliovirus Immunization Booster	X										
CBC w/diff		X				X		X	X	X	X
CMP		X				X		X	X	X	X
PT, aPTT		X									
Serum Pregnancy Test		X	X[c]								
Serum for LSQ and anti-tetanus toxoid IgG	X										
Whole blood for immunologic analysis and/or poliovirus titer (up to 10 mL, except for 4 mL on Day 1 and 6 mL on Day 14)	X	X				X	X	X	X	X	X (at 16 and 24 wks)[d]
Whole blood for immunologic analysis (not to exceed 3 mL/kg in a 24 hr period or 7 mL/kg in a 8 week period)	X[e]	X[e]					X[e]	X[e]	X[e]	X[f]	X (at 16 and 24 wks)[d, f]
Disease Evaluations											
MRI			X		X[g] (based upon date of enrollment)				X	X	X
CT Scan				X							
Biopsy				X							
Treatment											
PVSRIPO				X							

[a] Starting with Day 7 (Week 1), all tests and procedures have a 7-day plus or minus window.

[b] Daily after infusion until discharged from hospital

[c] Serum pregnancy test, if appropriate, within 48 hours of catheter placement and PVSRIPO

[d] At the discretion of the Principal Investigator, an additional blood sample at least 2 years post study drug infusion may be obtained.

[e] Whole blood draws for immunologic analysis may be taken (up to 36 mL) at the discretion of the Investigator, but should remain consistent with allowable volumes for pediatric patients depending upon the weight of the child (per Duke policy, the maximum blood volume drawn for research should not exceed 3 mL/kg in a 24 hr period or 7 mL/kg in an 8 week period).

[f] At 8, 16, and 24 weeks, up to 76.5 mL of whole blood may be drawn for immunologic analysis (please refer to Duke blood volume policy mentioned above).
[g] Prior to October 31, 2019, MRI to track Magnevist® containing infusate should have been obtained within 4 hours after completion of infusion. This MRI after the infusion was without gadolinium enhancement, but this MRI will not be performed on patients enrolled after October 31, 2019. All other MRIs in the study include gadolinium enhancement.
[h] Adverse events directly related to required screening procedures should be recorded.

12.1 Screening Examination

The screening examination will take place within 2 weeks before catheter placement. An informed consent must be signed by the patient and their legal guardian(s) before any study-specific screening procedure takes place. Within 6 months before receiving the PVSRIPO infusion, the patient should have received a PV booster, a serum test for LSQ and anti-tetanus toxoid IgG. The patient should also have whole blood drawn (up to 10 mL; red top tube(s)) for immunologic analysis and PV titer and an additional amount (up to 36 mL; yellow top ACD tube(s)) for other immunologic analysis during this time. Blood volumes drawn for immunologic analyses should not exceed 3 mL/kg in a 24 hour period or 7 mL/kg in an 8 week period, allowable per Duke policy. At the discretion of the investigator, blood volumes for immunologic analysis should be adjusted depending upon the weight of the child, in order to be consistent with Duke policy.

Pre-treatment evaluations within 2 weeks before catheter placement to determine eligibility and as a baseline will include the following, unless otherwise indicated:

- History and physical exam, including a full neurologic assessment and KPS or Lansky, within 2 weeks of catheter placement and within 5 days of PVSRIPO infusion
- Laboratory Evaluations:
    - CBC with differential
    - CMP
    - PT, aPTT
    - Beta-HCG, if appropriate (within 48 hours of catheter placement and PVSRIPO)
    - Anti-tetanus toxoid IgG (within 6 months of catheter placement and PVSRIPO)
    - LSQ (within 6 months of catheter placement and PVSRIPO)
    - Whole blood (up to 10 mL) for immunologic analysis and poliovirus titer
    - Whole blood (up to 36 mL, but not to exceed 3 mL/kg in a 24 hour period or 7 mL/kg in an 8-week period) for immunologic analysis (volumes drawn will be adjusted at the discretion of the Investigator, but will be based on the weight of the child to be consistent with Duke policy)
- Baseline MRI of the brain within 5 days of starting treatment

If a subject does not receive PVSRIPO, minimal records regarding the subject and the reason for screen failure will be retained in the study database.

12.2 Run-In Period

Not applicable.

12.3 Treatment Period

Day 0

- Biopsy and catheter placement
- After obtaining tissue for the standard clinical pathologic testing, up to three additional core biopsies will be obtained for molecular genetic tests (Section 9.1.4)
- CT of the brain to confirm catheter placement ≥ 1 cm from the ventricles prior to beginning infusion
- PVSRIPO infusion (infusion may be same day or within a +1 day window)

12.4 Follow-up Period

Day 1 post infusion

- History and physical exam, including a full neurologic assessment, to be performed **daily** until discharged from the hospital
- CBC with differential
- CMP
- Whole blood (up to 4 mL) for immunologic analysis

Week 1 (+/- 1 week)
- History and physical exam, including a full neurologic assessment and KPS
- Whole blood (up to 10 mL) for immunologic analysis and PV titer
- Whole blood (up to 36 mL, but not to exceed 3 mL/kg in a 24 hour period or 7 mL/kg in an 8-week period) for immunologic analysis at the discretion of the Investigator

Week 2 (+/- 1 week)
- History and physical exam, including a full neurologic assessment and KPS
- CBC with differential
- CMP
- Whole blood (up to 6 mL) for immunologic analysis and PV titer
- Whole blood (up to 36 mL, but not to exceed 3 mL/kg in a 24 hour period or 7 mL/kg in an 8 week period) for immunologic analysis at the discretion of the Investigator

Week 4 (+/- 1 week)
- History and physical exam, including a full neurologic assessment and KPS
- MRI of the brain with gadolinium contrast
- CBC with differential
- CMP
- Whole blood (up to 10 mL) for immunologic analysis and PV titer
- Optional whole blood (up to 36 mL, but not to exceed 3 mL/kg in a 24 hour period or 7 mL/kg in an 8 week period) for immunologic analysis at the discretion of the Investigator

Week 8 (+/- 1 week)
- History and physical exam, including a full neurologic assessment and KPS
- MRI of the brain with gadolinium contrast
- CBC with differential
- CMP
- Whole blood (up to 10 mL) for immunologic analysis and PV titer
- Whole blood (up to 76.5 mL, but not to exceed 3 mL/kg in a 24 hour period or 7 mL/kg in an 8 week period) for immunologic analysis at the discretion of the Investigator

Weeks 16, 24, 32, 40, 48 (+/- 1 week)
- History and physical exam, including a full neurologic assessment and KPS
- MRI of the brain with gadolinium contrast
- CBC with differential
- CMP
- Whole blood (up to 10 mL) for immunologic analysis and PV titer (weeks 16 and 24 only)
- Whole blood (up to 76.5 mL, but not to exceed 3 mL/kg in a 24 hour period or 7 mL/kg in an 8 week period) for immunologic analysis (weeks 16 and 24 only) at the discretion of the Investigator

After week 48, patient will follow-up at the discretion of the treating physician.
- Whole blood (up to 10 mL) for immunologic analysis and PV titer (At the discretion of the Investigator, this blood sample, at least 2 years post-study drug infusion, may be obtained.)
- Whole blood (up to 76.5 mL, but not to exceed 3 mL/kg in a 24 hour period or 7 mL/kg in an 8 week period) for immunologic analysis (At the discretion of the Investigator, this blood sample, at least 2 years post-study drug infusion, may be obtained.)

Patients may not be treated with any other modality (other than bevacizumab per the 'special considerations' in Section 9.1.6) unless progressive tumor is noted or they are otherwise removed from the study. Patients will be considered off study upon tumor progression or upon treatment of the tumor with another modality. When subjects are considered off study, this indicates that subjects will no longer be obligated to undergo study-related tests and procedures, but the data described below will still be collected from these subjects as feasible. Subjects will be followed for serious adverse events for 30 days after coming off study. Collection of the following additional data from off study subjects will be performed, if possible, but is not mandatory and will not be considered a deviation if the data cannot be obtained. Subjects' medical records will be reviewed for the remainder of their life, in order to collect data on subsequent treatments, disease progression, tumor size/volume, and survival. Follow-up activity will be at the discretion of the treating physician.

12.5 End of Study

The study will be considered complete once enrollment has been met, follow-up procedures outlined in Section 12.4 have been conducted on all subjects, and data analysis is concluded. The study may also be terminated early for any reason by the PI-sponsor.

Subjects that are lost to follow-up will be documented in the patient record and in the 21 CFR Part 11 database. In the compliant database, the subject will be marked as "Patient Status Unknown," along with a corresponding explanation, if any.

12.6 Early Withdrawal of Subject(s)

12.6.1 Criteria for Early Withdrawal

Subjects may voluntarily withdraw from the study at any time. The Principal Investigator may also withdraw a subject from the study at any time based on his/her discretion. Reasons for PI-initiated withdrawal may include, but are not limited to the following:

- Non-compliance of the subject
- Administrative issues
- Disease progression

12.6.2 Follow-up Requirements for Early Withdrawal

Subjects should be seen in clinic or contacted at a minimum of every 3 months for 1 year.

12.6.3 Replacement of Early Withdrawal(s)

Subjects that withdraw from the study prior to receiving PVSRIPO infusion, either voluntarily or due to ineligibility, will be considered non-evaluable; those subjects will be replaced.

12.7 Study Assessments

12.7.1 Medical History

Standard medical history will be obtained and documented per institutional guidelines.

12.7.2 Physical Exam

Standard physical exam and neurological assessment will be conducted and documented per institutional and PRTBTC guidelines.

12.7.3 Radiographic Review

A CT will be obtained to confirm catheter placement prior to beginning the PVSRIPO infusion. A MRI of the brain without gadolinium enhancement will be obtained within 4 hours of

completing the PVSRIPO infusion to monitor distribution of the infusion. A MRI of the brain with gadolinium enhancement will be obtained at all subsequent visits.

Given that imaging following immunotherapy differs greatly from what is typically seen following chemoradiation treatment or treatment with anti-angiogenic compounds, evaluation of response using with Macdonald criteria or RANO criteria is not appropriate in this trial. Immunotherapy can trigger an inflammatory immune response that is observed on imaging. Distinguishing between the inflammatory immune response and progressive disease is difficult. Therefore, an exploratory objective of this study is to describe radiographic imaging post-PVSRIPO treatment. Another exploratory objective of this study is to describe changes in tumor volume based upon radiographic imaging. For that reason, subjects who have gone off study may continue to be followed for collection of MRI measurements. For the purpose of this objective, relative tumor volume is estimated from the MRI using the metric of multiplying the widest tumor diameter with the perpendicular height of the tumor in that same slice.

12.7.4 Laboratory Evaluations

The timing of laboratory assessments that will be obtained during the course of the study is given above in **Table 2.**

12.7.5 Correlative Assessments

- Anti-tetanus toxoid IgG titer within 6 months prior to treatment
- Up to 10 mL of whole blood for tests of immunologic assays and anti-PV antibody titer at day 1 and weeks 1, 4, 8, 16, and 24 after treatment. Up to 6 mL collection of whole blood at week 2 (day 14).
- Up to 36 mL of whole blood for tests of anti-tumor immune responses within 6 months before receiving PVSRIPO, and at weeks 1, 2, and 4 and up to 76.5 mL at weeks 8, 16, and 24 after treatment. At the discretion of the Principal Investigator, an additional blood sample at least 2 years post study drug infusion may be obtained (see **Table 2**). Because of the large volume of the whole blood draws taken for immune monitoring, it will be essential to carefully consider the weight of each patient when determining whether a blood draw may occur, or how much blood may be drawn at a given time point. In this pediatric population the maximum blood volumes are 3 mL/kg in a 24 hour period or 7 mL/kg in an 8 week period. **Table 3** contains predicted blood draw volumes that are required for planned study assessments.

Table 3. Schedule of Blood Draw Volumes

Maximum blood draw volumes (mL) [a]							
	Screening		**Treatment**				
Day	**Within 6 months (≈d -28)**	**Within 14 days prior to catheter (≈d -14)**	**1**	**7**	**14**	**28**	**56**
CBC & CMP		6	6		6	6	6
PT, aPTT		1.8					
Pregnancy		2					
LSQ, tetanus titer	6						
Polio titer & immunologic analysis	Up to 10 (red top tube)	Up to 10 (red top tube)		Up to 10 (red top tube)	Up to 6 (red top tube)	Up to 10 (red top tube)	Up to 10 (red top tube)
Whole blood immunologic analysis	Up to 36[b] (yellow top ACD tube)	Up to 36[b] (yellow top ACD tube)		Up to 36[b] (yellow top ACD tube)	Up to 36[b] (yellow top ACD tube)	Up to 36[b] (yellow top ACD tube)	Up to 76.5[b] (yellow top ACD tube)
Daily Total	**52**	**55.8**	**6**	**46**	**48**	**52**	**92.5**
Total 8 week volume (day -28 through +28): 259.8 mL							

[a] Volumes for standard lab tests are based on DUHS Clinical Labs volumes for patients age 8-15. For patients in the lower age range of this study and/or with a low body weight, lower pediatric volumes (for patients age 0-8) may be used. Some volumes may be higher for patients >15 years old.

[b] Volumes of whole blood for immunologic analysis will be reduced as necessary to not exceed 3 mL/kg in a 24 hr period or 7 mL/kg in an 8 week period.

13 SAFETY MONITORING AND REPORTING

The Principal Investigator is responsible for the identification and documentation of adverse events and serious adverse events, as defined below. At each study visit, the Principal Investigator or designee must assess, through non-suggestive inquiries of the subject or evaluation of study assessments, whether an adverse event (AE) or serious adverse event (SAE) has occurred.

13.1 Adverse Events

An AE is any untoward medical occurrence in a subject receiving study drug and which does not necessarily have a causal relationship with this treatment. For this protocol, the definition of AE also includes worsening of any pre-existing medical condition. An AE can therefore be any unfavorable and unintended or worsening sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of PVSRIPO, whether or not related to use of the PVSRIPO. Abnormal laboratory findings with or without clinical significance are collected according to CTCAE v.4.

From the time the subject has their first study-related procedure until their last study-related procedure, all AEs must be recorded in the subject medical record and adverse events case report form. Thus, AE directly related to study-specific and required screening procedures should be reported.

AEs will be assessed according to the CTCAE version 4. If CTCAE grading does not exist for an AE, the severity of the AE will be graded as mild (1), moderate (2), severe (3), life-threatening (4), or fatal (5).

Attribution of AEs will be indicated as follows:

- Definite: The AE is clearly related to the study drug
- Probably: The AE is likely related to the study drug
- Possible: The AE may be related to the study drug
- Unlikely: The AE is doubtfully related to the study drug
- Unrelated: The AE is clearly NOT related to the study drug

13.1.1 AEs of Special Interest

Not applicable.

13.1.2 Reporting of AEs

A database of all adverse events (not just those considered related to the study drug) will be maintained in 21 CFR Part 11 Compliant database. The event will be categorized by organ system, relationship to treatment, its grade of severity, and resolution. The Principal Investigator and study statistician will periodically review the collective adverse events with the intention of identifying any trends or patterns in toxicity. If any such trends are identified, depending on their severity and frequency, a protocol amendment will be considered.

13.2 Serious Adverse Events

An AE is considered "serious" if in the opinion of the investigator it is one of the following outcomes:

- Fatal
- Life-threatening
- Constitutes a congenital anomaly or birth defect
- A medically significant condition (defined as an event that compromises subject safety or may require medical or surgical intervention to prevent one of the three outcomes above).
- Requires inpatient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant incapacity or substantial disruption to conduct normal life functions.

13.2.1 Reporting of SAEs

All serious and unexpected AEs should be reported immediately to the Principal Investigator or designee. Only AEs that the Principal Investigator determines to be serious, unanticipated, and related or possibly/probably (i.e. more likely than not) related to the research must be reported to the Duke IRB. Those AEs will be submitted in the electronic IRB system, according to the following guidelines:

- Report within 24 hours of learning about any subject's death that was unanticipated and more likely related to the research than unrelated;
- Report within 5 business days of learning about any serious, unanticipated, and related or possibly/probably related AEs;
- Report within 10 business day of learning about any other unanticipated problem or event that was more likely related to the research than unrelated.

SAEs must be reported to the Sponsor or their designee within 24 h of learning of the event whether or not the SAE is considered to be related to the study drug. Applicable forms provided by the Sponsor or their designee must be transmitted via secure email to pv_istari@klserv.com. The Sponsor must report to the FDA, in an IND safety report, any suspected adverse reaction that is both serious and unexpected. Before submitting this report, the sponsor needs to ensure that the event meets all three of the definitions contained in the requirement:

- Suspected adverse reaction (i.e. there is a reasonable possibility that the drug caused the AE)

- Serious
- Unexpected

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

The Sponsor is required to report to the FDA all IND Safety reports in writing within 15 days (7 days for unexpected fatal or life-threatening suspected adverse reaction). The FDA Form 3500A can be found on the FDA website, www.fda.gov. All other AEs will be reported to the FDA in the Annual Report.

13.3 Emergency Unblinding of Investigational Treatment
Not applicable.

13.4 Other Reportable Information
Not Applicable.

13.5 Special Warnings and Precautions
Not Applicable.

13.6 Safety Oversight Committee (SOC)
The Duke Cancer Institute SOC is responsible for annual data and safety monitoring of DUHS sponsor-investigator Phase I and II, therapeutic interventional studies that do not have an independent Data Safety Monitoring Board (DSMB). The primary focus of the SOC is review of safety data, toxicities and new information that may affect subject safety or efficacy. Annual safety reviews include but may not be limited to review of safety data, enrollment status, stopping rules if applicable, accrual, toxicities, reference literature, and interim analyses as provided by the Principal Investigator. The SOC in concert with the DCI Monitoring Team (see Section 14.1 for Monitoring Team description) oversees the conduct of DUHS cancer-related, sponsor-investigator greater-than-minimal-risk intervention studies that do not have an external monitoring plan, ensuring subject safety and that the protocol is conducted, recorded and reported in accordance with the protocol, standing operating procedures (SOPs), Good Clinical Practice (GCP), and applicable regulatory requirements.

13.7 External Data and Safety Monitoring Board (DSMB)
Due to a potential conflict of interest, an external DSMB-Plus was created for the Phase I clinical trial performed at Duke University Medical Center. The current study will continue to be monitored by the same DSMB-Plus. The external DSMB-Plus will be responsible for safeguarding the interests of trial subjects and assessing the safety of the interventions during the trial. The DSMB-Plus will provide recommendations about stopping or continuing enrollment in the trial. To contribute to enhancing the integrity of the trial, the DSMB-Plus may also formulate recommendations relating to the selection, recruitment, and retention of subjects and their management. Additional details regarding the responsibility of the DSMB-Plus and its chair may be found in the charter document.

14 QUALITY CONTROL AND QUALITY ASSURANCE

14.1 Monitoring
The Duke Cancer Institute (DCI) Monitoring Team will conduct monitoring visits to ensure subject safety and to ensure that the protocol is conducted, recorded, and reported in accordance with the protocol, standard operating procedures, good clinical practice, and applicable regulatory requirements. The DCI Monitoring Team has agreed to conduct monitoring of the first three subjects enrolled following completion of their 4-week follow up visit. This monitoring of eligibility is in conjunction with annual monitoring of accrual, regulatory, consenting, eligibility, conduct, safety, data quality, investigation product accountability, and biologic samples. This sequential patient monitoring for the first three patients will be followed by annual monitoring of 1-3 subjects until the study is

closed to enrollment and subjects are no longer receiving study interventions that are more than minimal risk. Results of the DCI monitoring may be shared with the Sponsor, Istari Oncology, Inc. In addition, the Sponsor may perform risk-based monitoring oversight visits for the study.

Additional monitoring may be prompted by findings from monitoring visits, unexpected frequency of serious and/or unexpected toxicities, or other concerns and may be initiated upon request of DUHS and DCI leadership, the DCI Cancer Protocol Committee, the Safety Oversight Committee (SOC), the Sponsor, the Principal Investigator, or the IRB. All study documents must be made available upon request to the DCI Monitoring Team and other authorized regulatory authorities, including but not limited to the National Institute of Health, National Cancer Institute, and the FDA. Every reasonable effort will be made to maintain confidentiality during study monitoring.

14.2 Audits

The Duke Office of Audit, Risk, & Compliance (OARC) office may conduct confidential audits to evaluate compliance with the protocol and the principles of GCP. The Principal Investigator agrees to allow the OARC auditor(s) direct access to all relevant documents and to allocate his/her time and the time of the study team to the OARC auditor(s) in order to discuss findings and any relevant issues.

OARC audits are designed to protect the rights and well-being of human research subjects. CTQA audits may be routine or directed (for cause). Routine audits are selected based upon risk metrics generally geared towards high subject enrollment, studies with limited oversight or monitoring, Investigator initiated Investigational Drugs or Devices, federally-funded studies, high degree of risk (based upon adverse events, type of study, or vulnerable populations), Phase I studies, or studies that involve Medicare populations. Directed audits occur at the directive of the IRB or an authorized Institutional Official.

OARC audits examine research studies/clinical trials methodology, processes and systems to assess whether the research is conducted according to the protocol approved by the DUHS IRB. The primary purpose of the audit/review is to verify that the standards for safety of human subjects in clinical trials and the quality of data produced by the clinical trial research are met. The audit/review will serve as a quality assurance measure, internal to the institution. Additional goals of such audits are to detect both random and systemic errors occurring during the conduct of clinical research and to emphasize “best practices” in the research/clinical trials environment.

14.3 Data Management and Processing

14.3.1 Case Report Forms (CRFs)

The electronic CRF (eCRF) will be the primary data collection document for the study and is developed in conjunction with statistical oversight. The CRFs will be updated in a timely manner following acquisition of new source data. Only the Principal Investigator, the study coordinator, the data management team, and the clinical trials manager are permitted to make entries, changes, or corrections in the eCRF.

An audit trail will be maintained automatically by the electronic CRF management system. All users of this system will complete user training, as required or appropriate per DCI requirements and other regulations.

14.3.2 Data Management Procedures and Data Verification

Access to electronic databases will be managed by the PRTBTC Data Manager.

Completeness of entered data will be checked automatically by the eCRF system, and users will be alerted to the presence of data inconsistencies. Additionally, the data management team and the statistical team will cross-reference the data to verify accuracy. Missing or implausible data will be brought to the attention of the Principal Investigator requiring appropriate responses (i.e.

confirmation of data, correction of data, completion or confirmation that data is not available, etc.).

The database will be reviewed and discussed prior to database closure, and will be closed only after resolution of all remaining queries. An audit trail will be kept of all subsequent changes to the data.

14.3.3 Study Closure

Following completion of the study, the Principal Investigator will be responsible for ensuring the following activities:

- Data clarification and/or resolution
- Accounting, reconciliation, and destruction/return of used and unused study drugs
- Review of site study records for completeness
- Shipment of all remaining laboratory samples to the designated laboratories

15 STATISTICAL METHODS AND DATA ANALYSIS

All statistical analysis will be performed under the direction of the statistician designated in key personnel. Any data analysis carried out independently by any investigator must be approved by the statistician and Sponsor before publication or presentation, in keeping with the executed clinical trial agreement(s).

15.1 Analysis Sets

All patients who receive PVSRIPO treatment will be included in all safety and efficacy analyses.

15.2 Patient Demographics and Other Baseline Characteristics

Socio-demographic and clinical characteristics of patients enrolled and treated on this study will be summarized. For categorical variables, frequencies and percentages will be provided. Means with standard deviations and medians/percentiles will summarize non-categorical variables.

15.3 Treatments

The number of patients that receive catheter placement will be calculated, as well as the number of patients who subsequently receive PVSRIPO infusion.

15.4 Primary Objective

The primary objective of this study is to confirm the safety of the selected dose of PVSRIPO treatment among pediatric patients with recurrent WHO Grade III malignant glioma (anaplastic astrocytoma, anaplastic oligoastrocytoma, anaplastic oligodendroglioma, anaplastic pleomorphic xanthoastrocytoma, ependymoma) or WHO Grade IV malignant glioma (glioblastoma, gliosarcoma).

15.4.1 Overview of Study Design and Monitoring

Patients will be treated with PVSRIPO, then carefully monitored for safety for at least 1 year after treatment. Of particular interest will be the incidence of AEs that occur during the first 14 days after PVSRIPO treatment and the inflammatory events that occur during the first year after PVSRIPO treatment. The experience with PVSRIPO treatment of adult patients with recurrent WHO grade IV malignant glioma suggests that a patient remains at risk, during the first 12 months post-PVSRIPO treatment, of demonstrating neurologic or radiographic signs suggestive of an inflammatory reaction secondary to the immune response triggered by PVSRIPO that requires a dose of dexamethasone above 4 mg a day. As described in Section 9.1.6, such patients will be treated with bevacizumab 7.5 mg/kg IV every 3 weeks if possible, or alternatively corticosteroids or surgery.

Two sets of safety monitoring guidelines are proposed, with one focused on acute toxicity (i.e. occurring during the first 14 days after PVSRIPO administration), and one focused on long-term issues.

Acute Toxicity Monitoring Guidelines: Monitoring rules based upon the occurrence of an unacceptable toxicity, as defined in Section 9.1.5, during the first 14 days after PVSRIPO administration are shown below in **Table 4**.

If the number of patients with an unacceptable toxicity surpasses the noted thresholds, accrual will be suspended. Data will be carefully reviewed to determine if accrual should be terminated, continued without modification, or continued with appropriate modification (e.g. PVSRIPO dose reduction).

Table 4. Acute Toxicity Monitoring Rules.

# of Patients Accrued	Accrual Suspension Threshold
0 – 6	≥2 patients with unacceptable toxicity
7-9	≥3 patients with unacceptable toxicity
10-12	≥4 patients with unacceptable toxicity

The probability of study suspension and the expected number of treated patients before study suspension is tabulated below in **Table 5** as a function of the true underlying probability of an unacceptable toxicity.

Table 5. Probability of study suspension.

True Underlying Probability of a Unacceptable Toxicity within 14 days of PVSRIPO administration	Probability of Accrual Suspension	Expected Number of Patients to be Accrued
0.05	0.030	11.8
0.10	0.124	11.1
0.15	0.262	10.2
0.20	0.411	9.2
0.25	0.560	8.1
0.30	0.681	7.2
0.35	0.792	6.2
0.40	0.873	5.4

***Long-term Monitoring*:** If any patient experiences an inflammatory reaction that does not improve within 18 weeks of commencing bevacizumab or steroid treatment, the inflammatory reaction will be considered “irreversible.” If a patient experiences “irreversible” inflammatory reaction, further enrollment of patients on the PVSRIPO trial will be temporarily interrupted and data on all previously treated patients will be carefully reviewed to determine if the study needs modification. Options may include dose reductions in future patients (next PVSRIPO dose would be 3.3 x 10^6 TCID50), modification of the approach to treating a patient with an inflammatory reaction, study termination, or accrual continuation without modification.

The FDA and/or the DSMB for this study may be part of this decision-making in the event that there are unacceptable toxicities or inflammatory issues.

15.4.2 Primary Objective Analyses

Percentage of patients that experience unacceptable toxicity during the 14-day period post-PVSRIPO treatment will be calculated.

The proportion of patients who require low-dose bevacizumab or steroid treatment due to an inflammatory reaction secondary to an immune response to PVSRIPO treatment will be calculated. In addition, the time to initiation of such treatment will be calculated and summarized using Kaplan-Meier methodology.

Adverse events experienced by protocol subjects from the time of catheter placement will be summarized in several forms to satisfy scientific and monitoring needs, as well as various regulatory reporting needs (e.g. FDA, DCI SOC, and ClinicalTrials.gov). These include a summary of the frequency of adverse events that are possibly, probably, or definitely related to protocol treatment tabulated by the maximum grade for each type of adverse event, as well as a summary of the frequency of adverse events regardless of attribution tabulated by the maximum grade for each type of adverse event.

15.5 Secondary Objective

The secondary objective is the estimation of overall survival (OS), which will be calculated as the time between PVSRIPO infusion and death, or last follow-up if the patient remains alive. The Kaplan-Meier estimator will be used to describe the survival of patients treated with PVSRIPO. Median OS will be estimated, as well as 6-, 12-, and 24-month OS. Analyses will be stratified by histologic grade.

15.6 Exploratory Objective

An exploratory objective of this study is to describe changes visualized on imaging due to intratumoral inoculation of PVSRIPO. Two components of the inflammatory reaction will be characterized: the enhancing lesion and the edema (FLAIR) area. Given that imaging following immunotherapy differs greatly from what is typically seen following chemoradiation treatment or treatment with anti-angiogenic compounds, evaluation of response using with Macdonald criteria or RANO criteria is not appropriate in this trial. Immunotherapy can trigger an inflammatory immune response that is observed on imaging. Distinguishing between the inflammatory immune response and progressive disease is difficult. Therefore, an exploratory objective of this study is to describe radiographic imaging post-PVSRIPO treatment.

15.7 Other Exploratory Objectives

An additional exploratory objective is to describe changes in tumor volume based upon imaging. Subjects' MRIs will be followed for changes in tumor volume post-PVSRIPO treatment. Another exploratory objective is the assessment of immunologic changes stemming from the administration of PVSRIPO treatment. Peripheral blood will be collected at defined intervals for correlative immune monitoring studies in serum and in peripheral blood monocytes. These include (i) analyses of innate and inflammatory immune events (HMGB1, inflammatory cytokines, NK and NKT-cells and regulatory immune subsets (Tregs & MDSCs)); (ii) analyses of adaptive immune responses (lineage, maturation, induction and activation/functional status of tumor antigen-specific T-cells). A final exploratory objective is to identify genetic predictors of response or failure of response to treatment with PVSRIPO. The Cox model will explore the impact of genetic markers of survival time. With logistic regression, the association between genetic markers and being a long-term survivor will be explored.

15.8 Interim Analysis

See Section 15.4.1.

15.9 Sample Size Calculation

A maximum of 12 patients will be treated on this study.

16 ADMINISTRATIVE AND ETHICAL CONSIDERATIONS

16.1 Regulatory and Ethical Compliance

This protocol was designed and will be conducted and reported in accordance with the International Conference on Harmonization (ICH) Harmonized Tripartite Guidelines for Good Clinical Practice, the Declaration of Helsinki, and applicable federal, state, and local regulations.

16.2 DUHS Institutional Review Board and DCI Cancer Protocol Committee

The protocol, informed consent form, advertising material, and additional protocol-related documents must be submitted to the DUHS Institutional Review Board (IRB) and DCI Cancer Protocol Committee (CPC) for review. The study may be initiated only after the Principal Investigator has received written and dated approval from the CPC and IRB.

The Principal Investigator must submit and obtain approval from the IRB for all subsequent protocol amendments and changes to the informed consent form. The CPC should be informed about any protocol amendments that potentially affect research design or data analysis (i.e. amendments affecting subject population, inclusion/exclusion criteria, agent administration, statistical analysis, etc.).

The Principal Investigator must obtain protocol re-approval from the IRB within 1 year of the most recent IRB approval. The Principal Investigator must also obtain protocol re-approval from the CPC within 1 year of the most recent IRB approval, for as long as the protocol remains open to subject enrollment.

16.3 Informed Consent

The informed consent form must be written in a manner that is understandable to the subject population and their legal guardian(s). Prior to its use, the informed consent form must be approved by the IRB.

The Principal Investigator or authorized key personnel will discuss with the potential subject and their legal guardian(s) the purpose of the research, methods, potential risks and benefits, subject concerns, and other study-related matters. This discussion will occur in a location that ensures subject privacy and in a manner that minimizes the possibility of coercion. Appropriate accommodations will be made available for potential subjects and their legal guardian(s) who cannot read or understand English or are visually impaired. Potential subjects and their legal guardian(s) will have the opportunity to contact the Principal investigator or authorized key personnel with questions, and will be given as much time as needed to make an informed decision about participation in the study.

Before conducting any study-specific procedures, the Principal Investigator must obtain written informed consent from the subject and/or their legal guardian(s). The original informed consent form will be stored with the subject's study records, and a copy of the informed consent form will be provided to the subject and their legal guardian(s). The Principal Investigator is responsible for asking the subject and their legal guardian(s) whether the subject wishes to notify his/her primary care physician about participation in the study. If the subject agrees to such notification, the Principal Investigator will inform the subject's primary care physician about the subject's participation in the clinical study.

16.4 Privacy, Confidentiality, and Data Storage

The Principal Investigator will ensure that subject privacy and confidentiality of the subject's data will be maintained. Research Data Security Plans (RDSPs) will be approved by the appropriate institutional Site Based Research group.

To protect privacy, every reasonable effort will be made to prevent undue access to subjects during the course of the study. Prospective participants will be consented in an exam room where it is just the research staff, the patient and his family, if desired. For all future visits, interactions with research staff (study doctor and study coordinators) regarding research activities will take place in a private exam room. All research related interactions with the participant will be conducted by qualified research staff who are directly involved in the conduct of the research study.

To protect confidentiality, subject files in paper format will be stored in secure cabinets under lock and key accessible only by the research staff. Subjects will be identified only by a unique study number and subject initials. Electronic records of subject data will be maintained using a dedicated 21 CFR Part 11 compliant database, which is housed in an encrypted and password-protected file on a secure network drive. Access to electronic databases will be managed by the PRTBTC Data Manager. The security and viability of the IT infrastructure will be managed by the DCI and/or Duke Medicine.

Upon completion of the study, research records will be archived and handled per DUHS HRPP policy. Subject names or identifiers will not be used in reports, presentations at scientific meetings, or publications in scientific journals.

16.5 Data and Safety Monitoring

Data and Safety Monitoring will be performed in accordance with the DCI Data and Safety Monitoring Plan.

16.6 Protocol Amendments

All protocol amendments must be initiated by the Sponsor and approved by the IRB prior to implementation. IRB approval is not required for protocol changes that occur to protect the safety of a subject from an immediate hazard. However, the Sponsor and/or Principal Investigator must inform the IRB and all other applicable regulatory agencies of such action immediately.

16.7 Records Retention

The Principal Investigator will maintain study-related records until the youngest child on study is 21 years old, or for at least six years after study completion, whichever is longer (Duke policy).

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